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NO. 9

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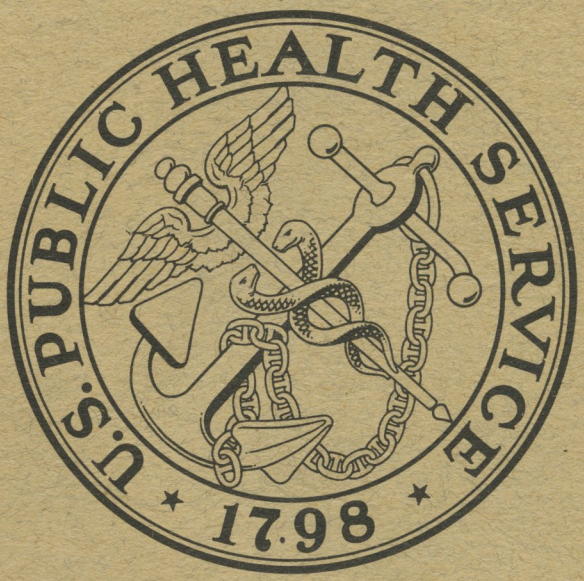

COMMUNICABLE DISEASE CENTER

TECHNICAL DEVELOPMENT DIVISION

SAVANNAH, GEORGIA

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INSECTICIDE INVESTIGATIONS BRANCH

R. W. Fay, E. L. Cole, A. J. Buckner

Effects of Surfaces and Household Cleaning Operations on the Residual Toxicity of DDT Spray Formulations

Tests on the comparative residual effectiveness of deposits from DDT-xylene emulsion, DDT-kerosene solution, and water-wettable DDT suspensions on various types of surface materials were previously reported (Summary of Activities No. 7, 3rd Quarter, 1946) for the initial 14 weeks after spray application. These tests have continued over an 8-month period and the summary results are shown in table 1.

Panels were sprayed with 200 mg. DDT per sq. ft. in all tests. The DDT-xylene emulsion was tested on three general types of surfaces, namely: Exterior surfaces represented by rusty and new samples of both sheet metal and metal screen, cement, dry bamboo, bark, and palmetto thatch; fabric surfaces represented by rayon, nylon, and celanese; and interior surfaces represented by tile, glass, plastics, and both shellacked and waxed wood.

The DDT-kerosene solution was tested on panels of new metal screen, rusty sheet metal, plexiglass, and green and dry bamboo. A single water-wettable powder was used to formulate all test suspensions, and tests were made on new sheet metal, glass, dry and green bamboo, tile, bark, palmetto thatch, and simulated adobe. Plain pine plywood was used as a standard surface in all studies.

In considering the results obtained with the DDT-xylene emulsion,

Table 1. Twenty-four-hour mortalities (percent) of adult A. quadrimaculatus females after 60-minute exposures to 200 mg. DDT per square foot from application of different formulations of DDT.

Age of Residues (Months)	Type of Spray														
	DDT-xylene Emulsion					Water-wettable DDT					DDT-kerosene Solution				
	1	2	4	6	8	1	2	4	6	8	1	2	4	6	8
Type Surface															
Pine plywood	93	89	91	81	64	84	-	62	53	-	84	78	77	69	58
Dry Bamboo	96	93	100	90	86	99	81	64	98	80	72	73	73	56	39
Rusty Sheet Metal	98	92	81	85	76	-	-	-	-	-	77	80	85	78	73
Rusty Metal Screen	99	98	99	93	78	79	71	42	25	-	-	-	-	-	-
Bark	-	90	86	87		77	64	54	88	68	-	-	-	-	-
New Sheet Metal	84	79	64	54	57	94	84	70	88	70	-	-	-	-	-
Glass	77	77	77	71	48	90	78	70	92	75	-	-	-	-	-
Tile (glazed)	78	72	77	84	58	90	78	70	76	60	-	-	-	-	-
Palmetto Thatch	-	41	82	75		77	64	54	88	68	-	-	-	-	-
New Metal Screen	98	90	79	46	38	-	-	-	-	-	75	62	49	24	-
Plexiglass	50	56	70	59	34	-	-	-	-	-	81	77	76	54	44
Cement	55	37	35	31	-	98	87	84	90	58	-	-	-	-	-
Mud	-	-	-	-	-	12	4	-	-	-	-	-	-	-	-
Green Bamboo	-	-	-	-	-	82	71	63	90	80	80	70	53	38	36
Shellacked Wood	-	74	70	23	-	-	-	-	-	-	-	-	-	-	-
Waxed Wood	-	21	37	19	-	-	-	-	-	-	-	-	-	-	-
Nylon	79	46	32	64	39	-	-	-	-	-	-	-	-	-	-
Rayon	78	58	54	45	44	-	-	-	-	-	-	-	-	-	-
Celanese	47	39	46	65	39	-	-	-	-	-	-	-	-	-	-

the surfaces were divided into three groups: (1) The dry bamboo, rusty sheet metal, rusty metal screen, and bark had residual toxicities equal to or better than the standard plywood; (2) the new sheet metal, glass, tile and palmetto thatch were three- to four-fifths as efficient as the standard; and (3) the new metal screen, plexiglass, cement, nylon, rayon, celanese, shellacked wood and waxed wood were three-fifths or less as efficient as the standard.

It is interesting to note that all the surfaces in the first group were relatively rough surfaces which might aid in giving a good spray coverage and adherence. All of the second group, however, had very smooth continuous surfaces which made it difficult to obtain good coverage as the emulsion spray separated into discrete droplets. In the third group the surfaces were either smooth and discontinuous such as the plastic fabrics and the new metal screen, or else surfaces such as shellac, wax, or plexiglass which might be temporarily softened and penetrated by the xylene in the emulsion, and later, upon drying, trap a portion of the DDT below the surface. The low residual effectiveness of the spray application on cement may well be due to the high porosity of this surface.

The DDT-kerosene deposits showed high effectiveness on rusty sheet metal, progressively less on pine plywood, dry bamboo, and plexiglass, and inferior effectiveness on the green bamboo and new metal screen.

The water-wettable DDT deposits showed definite superiority to the DDT-xylene deposits on the absorbent surface, cement, and somewhat better results on the smooth surfaces of glass and new metal. The effectiveness

of water-wettable DDT was less on the plywood, palmetto thatch, and on the dry and green bamboo, and definitely inferior on rusty metal screen, and bark surfaces. No residual effectiveness was obtained on simulated adobe, though visual evidence indicated an adequate treatment.

In addition to tests on the effectiveness of deposits from emulsions, solutions and suspensions of DDT, tests have been made on DDT incorporated in wallpaper and paints during the preparation of these materials. On the basis of the manufacturer's recommendations, the experimental tests were initiated to evaluate a direct comparison between samples of the impregnated wallpaper and a regular spray application of 200 mg. DDT per sq. ft. Chemical analysis demonstrated only 100 mg. DDT per sq. ft. in the impregnated wallpaper and it has not been ascertained if this amount of DDT represents a limit of DDT impregnation in the wallpaper. Kemtone water-paint was mixed with water-wettable DDT powder so as to obtain deposits of 400, 800, and 1600 mg. DDT per square foot, and with technical DDT to obtain deposits of 200, 400, and 800 mg. DDT per square foot as preliminary tests demonstrated that lower concentrations of DDT so mixed had little residual effectiveness. These impregnated paints were compared with a standard DDT-xylene emulsion application of 200 mg. DDT per square foot on ordinary Kemtone paint.

The wallpaper with impregnated DDT had considerably less residual effectiveness than the manufacturer's assertion and compared unfavorably to ordinary wallpaper with a DDT spray application (table 2). Water-wettable DDT powder incorporated in the Kemtone paint was less effective than the

Table 2. Twenty-four-hour mortalities (percent) of adult female A. quadrimaculatus after 60-minute exposures to DDT incorporated in wallpaper and paint and DDT applied as an emulsion spray.

Type of Surface	Mg. DDT per sq. ft.	Age of Deposits (weeks)				
		7	19	23	29	33
DDT in wallpaper	100	--	12	70	37	20
DDT on wallpaper	200	98	98	95	92	89
Water-wettable DDT in Kemtone	400	24	58	36	27	23
	800	25	68	39	24	18
	1600	17	57	55	44	45
Technical DDT in Kemtone	200	41	51	47	10	46
	400	52	59	44	38	60
	800	38	58	45	34	59
DDT on Kemtone	200	74	50	65	50	58

technical DDT incorporated in the paint. DDT incorporated in the Kentone was not as effective as a regular DDT emulsion application applied to the paint as a spray.

The study on the effect of house-cleaning and maintenance operations upon the deposits of DDT on treated surfaces has been described (Summary of Activities No. 7, 3rd Quarter, 1946) for dry-cleaning, laundering and ironing of fabrics. This study was extended to consider the effects of brushing and vacuuming over-stuffed furniture, the dusting of wood and wallpaper, and the use of paste cleaner on wallpaper, and of wax on shellacked wood and linoleum. As controls, duplicate sets of panels were sprayed, but did not receive any surface manipulations.

In a comparison of three types of manipulations on mohair, dry-cleaning with mineral spirits had the most effect, brushing somewhat less, and vacuuming the least deleterious effects upon DDT residual effectiveness (table 3). The mineral spirits, a solvent for DDT, readily removed it from the fabric. The initial vacuuming of mohair probably removed all DDT not firmly adhering to the fabric and subsequent treatments had little effect in reducing residual toxicity. Vigorous brushing, however, apparently removed more DDT each time it was done.

Dusting caused a gradual decrease in the residual toxicity of DDT on shellacked wood and wallpaper. Paste wallpaper cleaner rapidly removed the DDT deposit from wallpaper.

Waxing prior to spray application greatly decreased the residual effectiveness of DDT sprays on shellacked wood, but increased slightly the

Table 3. Twenty-four-hour mortalities (percent) of adult female A. quadrimaculatus from 60-minute exposures to residues of 200 mg. DDT per square foot which had been subjected to various surface treatments.

Type Surface	Surface Treatment	Number of Treatments										
		0	1	2	3	4	5	6	7	8	9	10
Mohair	None	98	86	88	90	91	88	80	82	82	84	72
	Dry-clean	93	27	8	--	--	--	--	--	--	--	--
	Brushing	72	81	53	38	22	23	--	--	--	--	--
	Vacuuming	100	77	74	75	73	72	62	41	56	28	10
Shellacked Wood	None	70	50	37	--	--	--	--	--	--	--	--
	Dusting	32	19	13	--	--	--	--	--	--	--	--
Wallpaper	None	99	98	98	99	95	85	87	92	89	--	--
	Dusting	92	77	53	40	38	30	24	32	37	--	--
	Paste Cleaner	86	59	38	22	13	--	--	--	--	--	--
Wood (plain)	None	70	50	37	42	40	--	--	--	--	--	--
Wood (waxed)	None	21	37	28	13	--	--	--	--	--	--	--
	Wax, Polish	17	13	--	--	--	--	--	--	--	--	--
Linoleum (plain)	None	1	1	--	--	--	--	--	--	--	--	--
Linoleum (waxed)	Wax, Polish	15	9	--	--	--	--	--	--	--	--	--

toxicity of the DDT deposits on linoleum. In the case of the wood, the emulsion apparently readily penetrated the wax and more DDT was lost below the surface than in the case of plain wood. On the other hand, in the case of the linoleum, the waxed surface was more impervious to the emulsion than that of plain linoleum which has been shown to be readily penetrated by DDT-xylene emulsion in previous work. In both instances, rewaxing caused a decrease in residual effectiveness as the DDT deposits were probably further covered by the wax layer.

Laboratory House Fly Control Studies

The effectiveness of deposits of 50-, 100-, and 200-mg. DDT per square foot against the house fly have been reported for the initial 22-week period after spray application (Summary of Activities No. 7, 3rd Quarter, 1946). A summary report of the results of this experiment over a ten-months period is now shown in table 4.

It is evident from these results (table 4) that satisfactory kills of the male flies are obtained in the laboratory with all dosages within the range of 50- to 200-mg. DDT per square foot; but for the more resistant female flies, the 50-mg. DDT per square foot application was inadequate after two months. The deposits of 200 mg. DDT per square foot were only slightly superior to the deposits of 100 mg. per square foot at four months after spray application.

The residual effectiveness of Dycore DDT-byproduct combinations[1/] was

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1/ A product of Hercules Powder Co., Wilmington, Delaware.

Table 4. Twenty-four-hour mortalities (percent) of adult house flies after 30-minute exposure periods to 50-, 100-, and 200-milligrams of DDT per square foot at indicated times after spray application.

Time after spraying (Months)	24-hour mortality (percent) from DDT deposits (mg. per sq. ft.)					
	50		100		200	
	M	F	M	F	M	F
1	100	100	97	97	100	99
2	100	95	100	98	100	100
4	99	17	96	67	100	83
6	88	18	99	51	98	43
8	--	--	96	39	86	49
10	--	--	97	25	94	42

Table 5. Twenty-four-hour mortalities (percent) of adult female M. domestica after 15- and 30-minute exposure periods to deposits of 200 mg. DDT (from Dycoro byproduct) per square foot at definite times after spray application.

Test Insecticide	Exposure Period	Age of Residues (weeks)						
		1	4	8	12	16	20	24
25-percent Dycoro	15 min.	35	91	55	65	74	57	23
	30 min.	39	99	99	100	98	96	75
35-percent* Dycoro	15 min.	36	97	94	96	98	98	86
	30 min.	80	99	99	100	99	99	100
35-percent** Dycoro	15 min.	99	98	97	100	94	93	—
	30 min.	100	94	94	100	100	100	—

*First tested at one week of age.

**Tested at 1, 2, 3, and 7 days during the first week, only the 7th day being reported here.

reported in relation to A. quadrimaculatus mosquitoes (Summary of Activities No. 8, 4th Quarter, 1946). The results with M. domestica as the test insect can now be summarized for a 24-week test period (table 5).

The Dycoro byproducts, as previously described, tend to remain as super-saturated droplets when deposited in spray applications and show a low initial mortality. By the end of the first two weeks after spray application, however, the residual effectiveness of the deposits showed marked increase. The 35-percent-Dycoro byproduct has shown slight superiority over the 25-percent-Dycoro byproduct when the two insecticides are compared at equal rates of application.

Comparative tests were made on a series of water-wettable DDT powders, containing varying percentages of DDT and inert carrier, which were supplied by various manufacturers. All powders were formulated to give sprays containing 5 percent DDT and were applied and tested with deposits of 200 mg. DDT per square foot. A standard DDT-xylene emulsion application was utilized as a control. The results of these tests (table 6) have been coded as to commercial manufacturers but showed considerable variation in the effectiveness of different brands of water-wettable powders.

Comparative Tests of Some Potential Residual Insecticides against Adult Flies and Mosquitoes

Laboratory investigations were made on several, recently-developed, organic insecticides having residual toxicity to determine their relative effectiveness against A. quadrimaculatus and M. domestica. The insecticides

Table 6. Twenty-four-hour mortalities (percent) of M. domestica adult females after 15-minute exposure periods to deposits of 200 mg. DDT per square foot from various water-wettable DDT suspensions.

Age of Residues (weeks)	Standard Emulsion 5% DDT	Sample Number 1 90% DDT	2 25% DDT	3 50% DDT	4 50% DDT	5 50% DDT	6 50% DDT	7 50% DDT	8 50% DDT
1	--	100	100	99	99	100	100	100	86
3	100	100	99	99	98	99	99	99	83
5	100	100	97	99	98	99	98	97	70
7	100	99	97	100	99	99	96	98	64
9	100	98	98	100	99	99	92	93	51
11	100	99	99	97	99	100	80	81	40
13	99	100	99	95	100	100	71	66	29
15	96	97	97	96	100	100	60	54	28
17	88	98	97	98	99	100	47	51	30
19	86	100	99	98	98	99	51	52	--
21	76	100	100	99	89	99	52	43	--
23	65	100	100	100	71	97	28	26	--
25	83	100	100	100	83	96	16	24	--
27	93	100	100	100	91	98	35	41	--
29	86	99	99	96	89	89	52	45	--
31	86	99	98	86	68	61	37	27	--
33	81	99	99	84	62	40	12	10	--
35	71	99	98	84	63	36	2	2	--
37	67	98	91	70	34	26	1	0	--

tested were xylene emulsions of DDT, benzene hexachloride (hexachlorcyclohexane), DDD, $C_{10}H_6Cl_8$, and a chlorinated camphene[2/] applied at the rate of 200 mg. per square foot on pine plywood.

In a comparison of figures 1 and 2, the following points might be emphasized: (1) For equal exposure periods, the insecticides have the same general order of effectiveness against both test insects. The only possible exception to this is benzene hexachloride which was tested only against A. quadrimaculatus. (2) In general, for the mosquito, A. quadrimaculatus, the insecticides show a gradual decrease of efficiency from the time of initial testing, but no marked break or drop in effectiveness. With the house fly, M. domestica, however, the insecticides tend to show a sharp break in effectiveness and a rapid drop in efficiency. In addition, certain insecticides, for example, DDD and the chlorinated camphene apparently have low initial toxicity and rise to a maximum efficiency several weeks after spray application. (3) The insecticides are also quite different in their initial toxicity to the two insects.

2/ Toxaphene, a product of Hercules Powder Company.

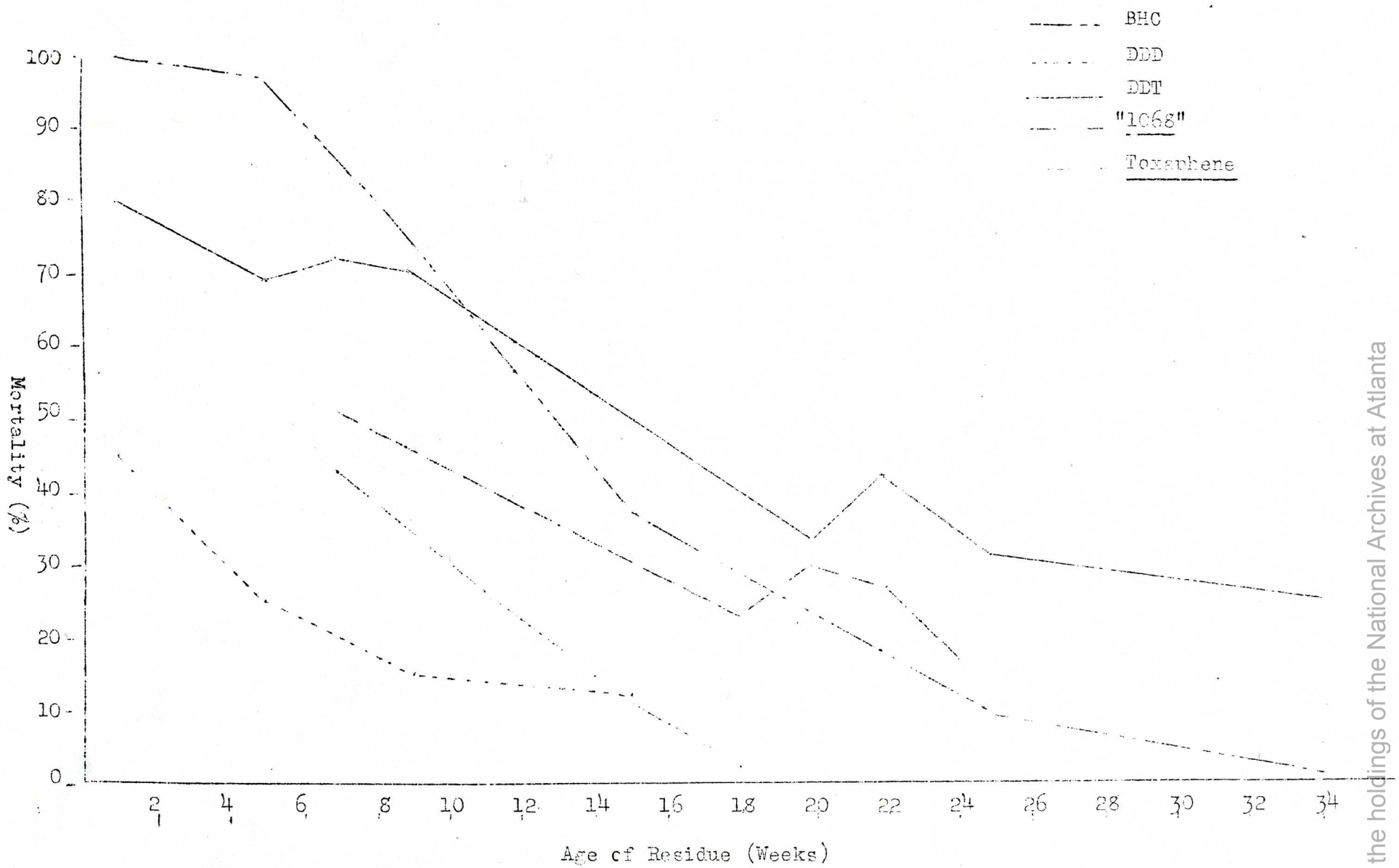

Figure 1. Twenty-four-hour mortalities (percent) of adult A. quadrimaculatus after 30-minute exposures at indicated times to deposits of 200 mg. various insecticides per square foot from a 5-percent xylene emulsion applied to plywood surfaces.

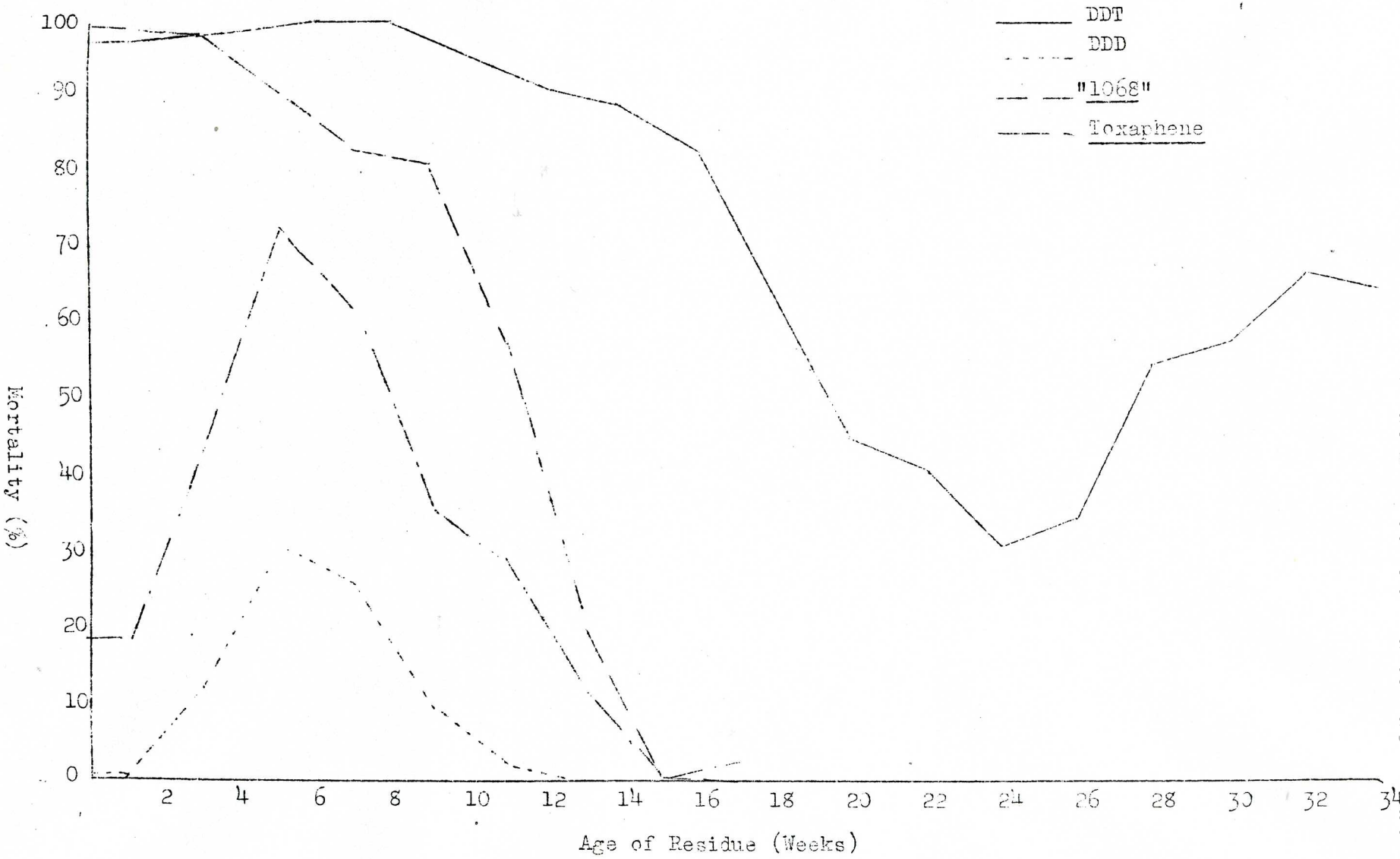

Figure 2: Twenty-four-hour mortalities (percent) of adult M. domestica after 30-minute exposures at indicated intervals to deposits of 200 mg. per square foot of various insecticides from a 5-percent xylene emulsion applied to plywood surfaces.

CONTROL METHODS AND EVALUATION BRANCH

K. D. Quarterman3/, R. H. McCauley, W. C. Baker
W. Mathis, J. G. Gillespie

Hookworm Control Investigations

Studies have been initiated to test various chemicals as hookworm larvicides for the purpose of developing practical soil treatment techniques for use as aids to hookworm control operations. Work during this quarter has been largely preliminary, particular emphasis having been directed to the development of testing techniques, the accumulation of various materials and equipment, and the preliminary testing of materials.

Two dogs with hookworm infections were obtained from the local pound and are being used as sources of hookworm eggs. The species of Ancylostoma involved has not yet been determined, but as both A. caninum and A. braziliense are common in this area, either or both may be involved. An effort will be made to obtain pure cultures of A. braziliense in these animals.

Laboratory cultures of larvae are obtained by mixing feces from infected dogs with sifted, heat sterilized, sandy loam placed in 7-inch diameter crystallizing dishes to a depth of 2 inches. After this preparation has been allowed to stand for one week or longer, apparently fully grown larvae are available.

The development of a method for obtaining an even distribution of

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3/ Reported for duty February 10, 1947.

larvae on the surface of the culture dish was considered important in testing to determine the radii of effectiveness of fumigants. In an effort to achieve even distribution of larvae, several methods of distributing the feces were tried. The best results were obtained by mixing enough water with the fecal material to render it semifluid, after which it was applied in small droplets over the surface of the soil. These droplets were then covered with a light sprinkling of dry wood ashes. Thus prepared, the container was covered and set aside for not less than one week in a room where the temperature remains constant at 85° F.

In order to take small uniform samples of infested soil from the culture dish, a device was designed and fabricated, on the principle of a post-hole digging tool, which was used to remove a cylindrical plug of soil with a surface area 1/4 square inch and a depth of two inches. In using this device, it was desirable to take a series of random samples at various distances from the point where the soil fumigant was injected. To facilitate this a metal disc was designed and made which contained holes at appropriate distances from the center through which the sampler could be thrust. The disc was marked in 10 segments numbered from 1 to 10. This device was then used in conjunction with a table of random numbers, by turning it so that the appropriate number coincided with a mark arbitrarily placed on the edge of the culture dish, the sample being taken through a hole at the desired distance from the center.

In extracting hookworm larvae from samples taken as described above, a Baermann apparatus was used. Tests were made to determine the amount of

water which should be drawn from this apparatus in order to recover all larvae present. It was found that the first 25 cc. of water usually contained 90 percent or more of the larvae present, while for practical purposes a drawing of 38 - 40 cc. would recover all larvae.

Preliminary tests were made by treating cultures with various chemical preparations according to the recommendations for soil nematode control of the respective manufacturers. To date, tests have been started with dichloropropane-dichloropropylene, methyl bromide, ethylene dibromide, and calcium cyanamide. The first of these, D-D, caused the elimination of all larvae within 4 days after treatment but is probably too toxic for general use. Results to date with methyl bromide have been unsatisfactory. Ethylene dibromide caused a considerable reduction but failed to produce a total kill of worms. Tests with calcium cyanamide suggest that this material may be of value, although much additional data is needed. Initial tests with this chemical have not been entirely satisfactory, possibly due to its slow action. In general, none of these materials have been tested sufficiently to form a basis for conclusions.

EVALUATION OF THERMAL-AEROSOL FOG GENERATOR AS AN APPARATUS FOR APPLYING RESIDUAL SPRAY

Equipment Development, Chemical Investigations, Insecticide Investigations, and Control Methods and Evaluation Branches

During recent months considerable publicity has been given throughout the country to the use of thermal-aerosol fog generators as an effective means of applying a space treatment of insecticide for controlling insect pests in recreation areas, on garbage dumps, on livestock, in orchards, on field crops, and in buildings. Field demonstrations of its use in treating buildings resulted in a few requests for adopting this method for treating homes on the extended malaria control program, although no factual data were available as to the residual qualities of the treatment. In March, an opportunity was open to evaluate this equipment with respect to the residual qualities obtained.

Equipment:

The applicator[4/] employed in the field tests consisted essentially of the following: a compact combustion chamber with positive-displacement-type air-pump to provide a gaseous product of 900° F. temperature at the discharge nozzle, a vane type solution pump with metering valve to regulate the flow of insecticide to the discharge nozzle, a 6 h.p. gasoline engine to drive the pumps, a 12-gallon capacity gasoline tank,

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4/ Todd Insecticidal Fog Applicator, manufactured by the Todd Shipyards Corp., New York.

suction and return hose for solution, solution tank and necessary accessories.

The gasoline engine was operated at 2450 r.p.m. so as to obtain 135 c.f.m. of air for combustion with an outlet temperature of 900° F. The solution pressure was adjusted for 25 psi during the tests and the metering valve set to provide the desired rate of discharge of insecticide. The gasoline pressure for the combustion chamber was maintained at 50 psi. The fuel rate during continuous operation was approximately 3 gallons per hour.

The solution-pump capacity was about 4 gpm with pressures up to 50 psi. A valve outlet for enabling use of the pump for straight pressure spraying by means of standard hose and spray guns was incorporated in the unit. Satisfactory supply for two spray guns was obtained in a test of this phase of the equipment.

The fog droplet size was regulated by adjusting the flow of insecticide to the discharge nozzle. The fog produced by the machine when the rate of flow of the insecticide was 10 gallons per hour, was described by the manufacturer as a "dry" fog, having an average particle size from 1 to 5 microns; an "intermediate" fog with particle size from 20 to 30 microns would be produced by increasing the rate of flow of insecticide to 30 gallons per hour; and a "wet" fog having a particle size from 50 to 60 microns would be secured by an insecticide flow of 40 gallons per hour. The figures on particle sizes supplied by the manufacturers were based on kerosene as a solvent.

The machine operated fairly well during the test. However, the metering valve performed erratically during formation of the "dry" fog. Clogging of the valve occurred several times when set at the 10 gph rate. Furthermore, the fuel nozzle and strainer for the combustion chamber had to be cleaned during test and it was necessary to clean the filter for the air pump to maintain satisfactory combustion. The unit had to be stopped on one occasion to re-prime the solution pump.

Testing Procedure:

Unoccupied housing units of concrete block construction were treated in the field tests. These units had cement floors; the interior concrete block walls had been painted with water paints several years previously; and the ceilings were of plaster wallboard. The bedrooms of these units were of uniform size, which would permit comparative evaluations of the treatments, which were applied to single bedrooms only, three-room units and five-room units. Evaluations of the treatments were made only in the bedrooms. The single rooms were expected to reveal the ability of the machine to apply a residual treatment in the room into which the insecticide was actually discharged, while the bedrooms of the three- and five-room units would indicate the residual which might result in rooms adjacent to the one into which the insecticide was discharged.

The three-room units consisted of a main room, which was subdivided by partial partitions to form a bathroom, kitchen and living room, and a bedroom which adjoined the living room. During treatment, all interior doors were left open and all exterior doors and windows were kept closed,

except one window of the living room through which the fog was discharged into the unit. This window was partially open during treatment and was closed immediately following treatment.

The five-room units were similar to the three-room units, except that three bedrooms adjoined the living room, two on one side and one on the other. Treatment of these five-room units was applied through one of the living room windows, as described above for the three-room units.

Two of each of the one-room units, three-room units, and five-room units, were treated with the "dry" fog, two of each were treated with the "wet" fog, and two three-room units were treated with the "intermediate" fog. A 10 percent concentrate of DDT in a mixture of kerosene and xylene was used. The amount of insecticide discharged into each unit was sufficient to apply approximately 200 mg. of DDT per square foot of exposed surface area, including walls, floors and ceilings, if the material were uniformly distributed over all surfaces. The amount of insecticide discharged into each unit was first calculated on the basis of the output of the machine and the time of operation. In each instance this was checked by measuring the contents of the insecticide tank, however, and, whenever necessary because of mechanical difficulties encountered, additional material was applied to bring up the amount of insecticide used to the correct quantity. Each of the treated units was kept closed for two hours following treatment, after which they were opened and aerated. In most instances, the fog had largely dissipated before the units were opened.

Three different types of evaluations of the residues applied by the

machine were planned: (1) chemically by quantitative analyses, (2) biologically by laboratory exposure of adult house flies (Musca domestica) to panels exposed in the bedrooms during treatment, and (3) biologically by the release of insectary-reared mosquitoes, Anopheles quadrimaculatus, into the treated bedrooms.

Chemical Evaluation

Procedure:

The dosage of DDT deposited on the walls, ceilings, and floors of the housing units tested was determined by collecting the DDT-oil droplets on coarse filter paper. The filter paper, which was cut into 7" x 6" strips, was attached to the walls, ceilings, and floors with masking tape. Five samples were taken from each wall, ceiling, and floor, and each set combined as one for purposes of analyses. After two hours of exposure, the strips of paper were removed and were trimmed to 6" x 6", so that an area of 1.25 square feet was sampled for each wall, ceiling, and floor.

The DDT on the filter paper was extracted with benzene for two hours in a Soxhlet extractor. The extracted DDT was then diluted to 100 milliliters in a volumetric flask, and aliquot portions were taken for analyses. The procedure used for analysis of DDT was a colorimetric procedure based upon nitration of DDT and addition of sodium-methylate to develop the color.

Results:

The deposition of DDT on the walls, ceilings, and floors of the various units treated are summarized in table 7. By far the greatest wall

Table 7. Recovery of DDT from houses treated with insecticidal fog. Application rate = 200 Mg. DDT/sq. ft.

Type of Housing Unit Treated	Type of Fog	Recovered Dosage, Mg. DDT/sq. ft.					
		Floor	Ceiling	Walls			
				N	S	E	W
Single Room Only	Dry	136	40	14	44*	25	5
	Wet	327	106	81*	23	27	22
Three Room Unit, Only Bedroom Tested	Dry	80	6	4	3	4	5
	Intermediate	88	4	3	3	3	4
	Wet	80	7	6	4	4	4
Five Room Unit, Only Bedrooms Tested							
Bedroom #1	Dry	72	22	4	5	5	5
Bedroom #2	Dry	108	19	8	9	9	8
Bedroom #3	Dry	114	23	8	6	6	7
Bedroom #1	Wet	363	7	3	11	5	4
Bedroom #2	Wet	175	6	4	5	4	8
Bedroom #3	Wet	238	7	10	4	4	5

*Wall was in direct line of discharge.

Note: Discharge was directed upward, so that ceiling and opposite wall were both in line of discharge.

recovery was obtained in the single rooms which received the fog directly. In these rooms, better recovery of DDT was obtained with the wet fog than with the dry fog. In rooms which were treated indirectly, much less DDT was recovered from the walls and little difference could be detected between the wet and dry fogs. It is noteworthy that in all cases, the floor received the majority of the applied DDT. The mass median diameter was 8 microns for the dry fog and 30 microns for the wet fog.

Laboratory Biological Evaluations

Procedure:

Coarse filter paper panels, size 3" x 12", were exposed in the bedrooms on the walls and ceilings during treatment. Previous experience has shown that filter paper can be expected to give results roughly comparable to wood panels. One panel was exposed on each of the four walls of the bedrooms and four panels were exposed on the ceilings of each bedroom, except in the case of the five-room units, where the ceiling panels were not placed in the bedrooms, but in the main living-room part of the unit in direct line of the discharge of the machine. The ceiling panels in the main living room were introduced to test the effectiveness of the generator in depositing residues at a distance of 30 ft. in direct line from the outlet. This was the maximum direct line distance available in the experimental plan. After two hours of exposure in the treated units, these panels were removed and inserted into a wooden framework to form a laboratory exposure chamber having one square foot of treated surface area.

The four panels from the walls of each room were used to make one chamber and those from the ceiling were used for another. Insectary-reared adult house flies, M. domestica, were exposed in these chambers for 15- and 30-minute periods, after which they were transferred to observation cages where the 24-hour mortalities of the females were noted. Tests with these panels were made one week and three weeks after treatment.

Results:

Table 8 summarizes the results obtained in the laboratory tests with the panels exposed in the rooms during treatment. Appreciable mortalities were obtained only with those panels which had been placed in the rooms into which the insecticide was discharged, verifying biologically the findings of the chemical analyses. The ceiling panels in the 5-room units, used in the biological tests, were located in a direct line with the generator outlet and received a heavy application as sufficient fog for the entire 5-room unit was administered through a single window. The ceilings of the bedrooms of the 3- and 5-room units should be closely comparable, since they were about the same distance from and in the same relative positions to the machine. The residues from the "wet" fog appear to be somewhat better than those from the "dry" fog treatment. In making the second check three weeks after treatment, the 15-minute exposure was omitted with those panels which had given no mortality in the check made one week after treatment.

Additional checks are planned on those panels which produced significant mortalities in the three-week check.

Field Biological Evaluations

Low temperatures have prevented the field evaluation of the treated rooms with insectary-reared adult mosquitoes, A. quadrimaculatus. These releases will be made as soon as weather conditions permit.

Conclusions

Chemical analyses and initial laboratory biological tests indicate that appreciable toxic residues are applied by this fog generating machine mainly in the room into which the insecticide is directly discharged and that these residues are only 10 to 15 percent as heavy as those which would be obtained with a regular hand sprayer application using the same quantity of insecticide. The residues applied on the walls and ceilings of rooms adjacent to and opening into the room into which the insecticide was discharged, were extremely low, averaging less than 10 mg. of DDT per square foot.

The fine particle size and thorough penetration of the fog into all cracks and crevices indicate that the machine would undoubtedly be very effective for applying indoor space treatments for immediate insect kills. While no tests were made on the treatment of outdoor spaces, it appears that this machine would function as well as other aerosol generating equipment, but would be subject to about the same limitations. In summation, the preliminary results obtained by chemical analyses and laboratory biological tests indicate that this machine is not as satisfactory as hand spraying for applying a residual house treatment. Further verification by field biological tests will be made when weather conditions are favorable for such work.

Table 8. 24-hour mortalities (percent) of adult female, M. domestica after 15- and 30-minute exposures to wet, dry, and intermediate fogs on filter paper surfaces.

Exposure (Minutes)	Time After Treatment (Weeks)	Wet Fog							Dry Fog							Inter-mediate Fog	
		1 Rm.		3 Rm.		5 Rm.			1 Rm.		3 Rm.		5 Rm.			3 Rm.	
		C	W	C	W	C	W	W	C	W	C	W	C	W	W	C	W
15	1	59	80	0	0	100*	0	0	40	50	0	0	80*	3	4	0	0
	3	90	25	-	-	100*	-	-	74	2	-	-	33*	-	3	-	-
30	1	96	99	0	0	100*	0	0	79	72	0	0	77*	0	0	0	0
	3	100	95	0	0	100*	0	0	100	42	0	0	83*	0	2	0	0

*Panels placed approximately 30 ft. from applicator, directly in line of discharge.

C = panels exposed on ceilings.
W = panels exposed on walls.

Field Biological Evaluations

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The fine particle size and thorough penetration of the fog into all cracks and crevices indicate that the machine would undoubtedly be very effective for applying indoor space treatments for immediate insect kills. While no tests were made on the treatment of outdoor spaces, it appears that this machine would function as well as other aerosol generating equipment, but would be subject to about the same limitations. In summation, the preliminary results obtained by chemical analyses and laboratory biological tests indicate that this machine is not as satisfactory as hand spraying for applying a residual house treatment. Further verification by field biological tests will be made when weather conditions are favorable for such work.

CHEMICAL INVESTIGATIONS BRANCH

W. R. Schmitz, L. B. Goette, S. L. Resnick

Penetration of DDT into Poplar Wood

Errata

In "Summary of Activities" No. 8, the effect of concentration of DDT in solution or emulsion on the penetration of DDT into poplar-wood surfaces was reported. The results given for the penetration of a 7-percent- and a 10-percent-DDT emulsion are in error. A DDT concentrate was mislabeled, and consequently, the 7-percent- and 10-percent-DDT emulsions prepared from this concentrate did not have the required amount of DDT.

The recovery of DDT at about 0.001 inch of the top surface should have been 41 percent and at about 0.006 inch of the top surface 65 percent for the 7-percent-DDT emulsion as compared to 50 percent at about 0.001 of an inch and 80 percent at about 0.006 of an inch for the 10-percent-DDT emulsion.

In general, DDT solutions and emulsions that were more concentrated gave better recovery of DDT at about 0.001 and 0.006 of an inch of the top surface than did the less concentrated solutions and emulsions. Particularly good recovery of DDT was obtained with the 10-percent-DDT emulsion, but at the application rate of 200 milligrams of DDT per square foot, the volume of emulsion was not sufficient to give good coverage of the surface. On poplar wood, 4 milliliters of liquid per square foot gave the best coverage without danger of "run-off."

Recovery of DDT at various depths in poplar wood

The penetration of 5 percent DDT in kerosene and 5 percent DDT in emulsion to various depths of poplar wood was determined to provide a standard for other tests conducted. Poplar wood panels, 3" x 12", were sprayed by a micro-sprayer at the rate of 50 milligrams of DDT per panel, and approximately 0.001, 0.006, 0.010, 0.025, and 0.040 of an inch of the top surface was removed. The amount of DDT recovered from each depth is shown graphically in figure 3. From the graph, it is apparent that after about 0.010 of an inch of the top surface has been removed, a straight line relationship exists between the recovery of DDT and the depth to which the DDT penetrates. It was necessary to remove about 1/8 of an inch of the top surface to insure complete recovery of DDT. Better recovery of DDT was obtained at each depth from the emulsion than from the kerosene solution.

All DDT which penetrated further than 0.001 of an inch of the top surface was probably of no value biologically. Within 0.001 of an inch of the top surface, the emulsion deposited about 38 percent of the applied DDT and the kerosene solution deposited about 30 percent of the applied DDT. Better recovery of DDT from the emulsion was possibly due to a more rapid rate of DDT crystallization from the emulsion than from the kerosene solution. The recovery of DDT at various depths of poplar wood is summarized in table 9.

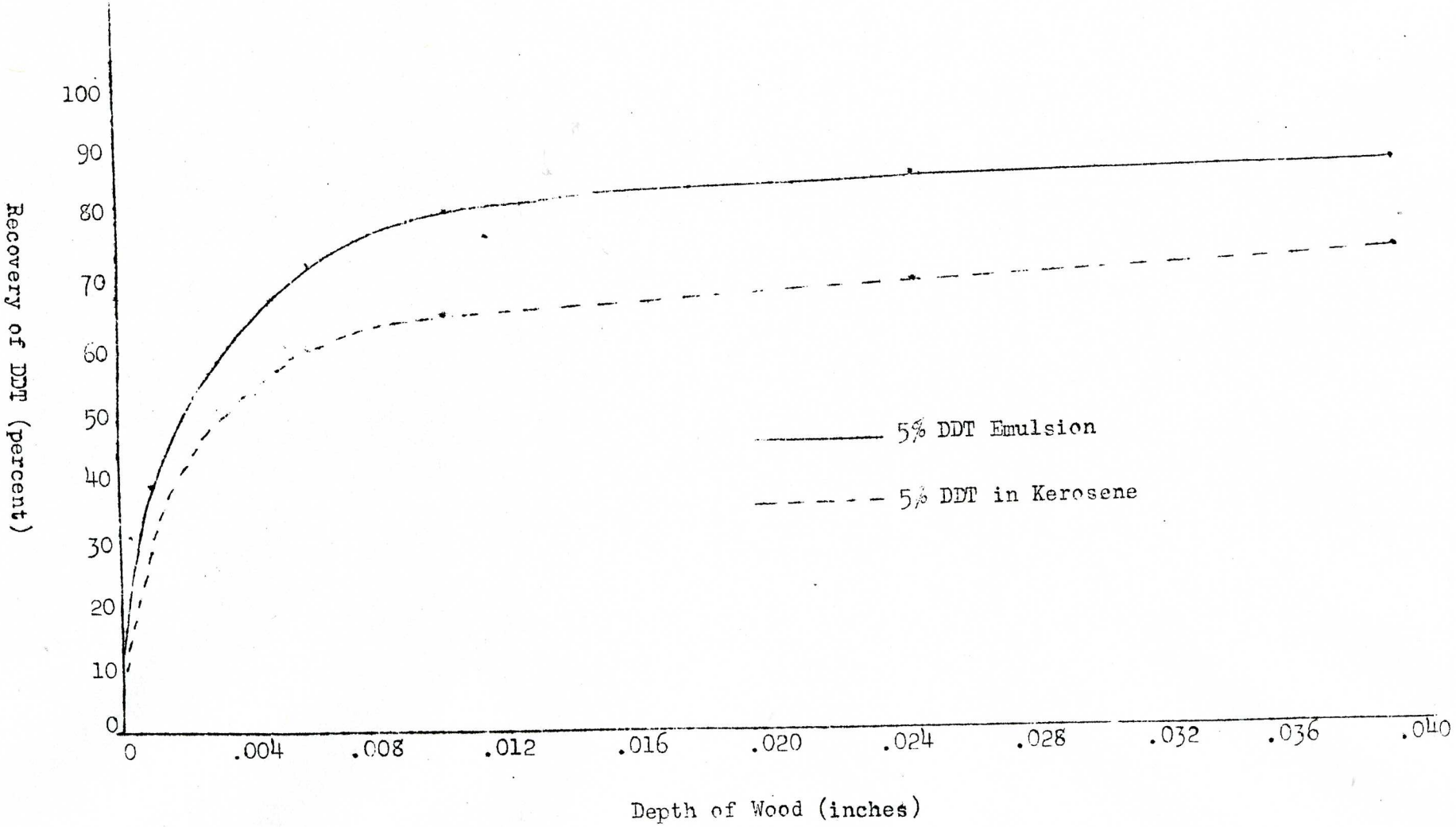

Figure 3. Penetration of DDT into poplar wood when applied at the rate of 200 miligrams per square foot.

Table 9. The cumulative recovery of DDT at various depths in poplar wood, which had been treated at the rate of 200 mg. of DDT per square foot.

Depth of wood removed, inches	5% DDT in Kerosene	5% DDT Emulsion
0.001 ± 0.0005	30%	38%
0.006 ± 0.001	60%	73%
0.010 ± 0.001	65%	81%
0.025 ± 0.001	70%	86%
0.040 ± 0.001	74%	88%

Note: Percentages are based upon the amount of DDT theoretically applied.

Effect of Solvent on Penetration of DDT into Poplar Wood

The penetration of DDT with various practical solvents was determined at about 0.001 and 0.006 of an inch of the top surface of poplar wood. A DDT concentrate was first prepared according to the following formula: 35 grams of DDT, 2 milliliters of an emulsifier (Triton X-100), and sufficient solvent to make a total volume of 100 milliliters. On a volume basis, one part of the concentrate was diluted with six parts of water and a 5-percent-DDT emulsion was prepared. Each panel was sprayed with one milliliter of emulsion or 50 milligrams of DDT.

Of the solvents tested, xylene, which was the most volatile, gave the best recovery of DDT at both 0.001 and 0.006 of an inch of the top surface. Xylene, cyclohexanone, and Solvesso #2 can be grouped as roughly four times more volatile than tetralin, PD 544-C, and Velsicol AR-50. The average recovery of DDT from the more volatile group of solvents was 32 percent at about 0.001 of an inch and 62 percent about 0.006 of an inch as compared to an average recovery of DDT of 25 percent at about 0.001 of an inch and 40 percent at about 0.006 of an inch for the less volatile group of solvents. Although tetralin and Solvesso #2 were exceptions, there was an indication that the more volatile the solvent, the better recovery of DDT that could be expected at the top surface. The recovery of DDT from different solvents is summarized in table 10.

Penetration of DDT into Different Woods

The penetration of DDT in a kerosene solution containing 5 percent DDT and a 5-percent-DDT emulsion was determined at about 0.001 and 0.006 of an

Table 10. Recovery of DDT from different solvents on poplar wood which had been treated at the rate of 200 mg. of DDT per square foot.

Solvent used in 5% DDT emulsion	Depth of poplar wood removed, inches	
	0.001 ± 0.0005	0.006 ± 0.001
Xylene	38%	73%
Tetralin	35%	47%
Cyclohexanone	31%	58%
Solvesso #2	27%	56%
PD-544-C	23%	39%
Velsicol AR-50	16%	35%

Note: Percentages are based upon the amount of DDT theoretically applied.

inch of the top surface of various woods. All of the woods had been air-dried, and probably contained about 12 percent moisture. Panels of white oak, longleaf pine, red gum, yellow poplar, a light-weight ash, Southern cypress, and white pine were prepared and each was sprayed with 50 milligrams of DDT. The woods listed above are in decreasing order of hardness and specific gravity, with white oak more than twice as hard and heavy as white pine. The number of growth rings per inch varied from 18 for longleaf pine to 9 for ash.

There appeared to be little correlation between the physical properties of the woods and the recovery of DDT at about 0.001 and 0.006 of an inch of the top surface. The average recovery of DDT from all woods was 25 percent at about 0.001 of an inch and 52 percent at about 0.006 of an inch for the kerosene solution containing 5 percent DDT as compared to 37 percent at about 0.001 of an inch and 70 percent at about 0.006 of an inch for the 5-percent-DDT emulsion. The recovery of DDT from different woods is summarized in table 11.

Effect of Drop Size on Penetration of DDT into Poplar Wood

The effect of drop size on the penetration of DDT was tested by spraying drops with a mass median diameter of 100 microns and pipetting drops with a mass median diameter of 3000 microns. Panels of poplar wood were treated with 5 percent DDT in kerosene and 5 percent DDT in emulsion at the rate of 50 milligrams of DDT per panel, and the penetration of DDT at about 0.001 and 0.006 of an inch of the top surface was determined.

The recovery of DDT from drops having a mass median diameter of 100

Table 11. Recovery of DDT at about 0.001 and 0.006 of an inch of the top surface of different woods which had been treated at the rate of 200 mg. of DDT per square foot.

Type of Wood	5% DDT in Kerosene		5% DDT Emulsion	
	0.001"±0.0005"	0.006"±0.001"	0.001"±0.0005"	0.006"±0.001"
White Oak	21%	57%	41%	72%
Longleaf Pine	17%	32%	35%	67%
Red Gum	34%	64%	33%	63%
Yellow Poplar	30%	60%	38%	73%
Ash	28%	48%	42%	72%
Southern Cypress	17%	40%	41%	74%
White Pine	31%	61%	29%	67%

Note: Percentages are based upon the amount of DDT theoretically applied.

microns was essentially the same as from drops having a mass median diameter of 3000 microns. When a 5-percent-DDT solution or emulsion was applied at the rate of 50 milligrams of DDT per panel, a continuous film was produced on the surface. Since the drops soon ran together after hitting the surface, the size of the applied drop had little meaning. The recovery of DDT from each drop size is summarized in table 12.

Table 12. Effect of drop size on recovery of DDT at about 0.001 and 0.006 of an inch of the top surface of poplar wood which had been treated at the rate of 200 mg. of DDT per square foot.

Mass median diameter of drops, microns	5% DDT in Kerosene		5% DDT Emulsion	
	0.001"±0.0005"	0.006"±0.001"	0.001"±0.0005"	0.006"±0.001"
100	30%	60%	38%	73%
3000	33%	60%	39%	79%

Note: Percentages are based upon the amount of DDT theoretically applied.

Summary

The penetration of a kerosene solution containing 5 percent DDT and a 5-percent-DDT emulsion was determined at various depths in poplar wood. Approximately 30 percent of the DDT applied from a kerosene solution was within 0.001 of an inch of the top surface as compared to about 38 percent of the DDT applied from a xylene emulsion. It was necessary to remove about 1/8 of an inch of the top surface of poplar wood to insure complete recovery of DDT.

The solvent used to make a DDT emulsion affected the penetration of DDT into poplar wood. Generally, better recovery of DDT was obtained with the

more volatile group of solvents. Of the solvents tested, a xylene emulsion gave the best recovery of DDT.

Applied at the rate of 200 milligrams of DDT per square foot, an increase in concentration of DDT in a solution or an emulsion gave better recovery of DDT. However, a 5-percent-DDT solution or emulsion gave better coverage of poplar wood surfaces.

The penetration of a kerosene solution containing 5 percent DDT and a 5-percent-DDT emulsion was determined on various woods. There was little correlation between the physical properties of the wood and the recovery of DDT.

The drop size of the applied DDT solution or emulsion had little effect on the penetration of DDT into poplar wood.

Poplar wood which had been sprayed with water, and after 15 minutes was sprayed with DDT, gave better recovery of DDT than dry poplar wood.

In all tests, better recovery of DDT was obtained from 5 percent DDT in a xylene emulsion than from 5 percent DDT in kerosene solution.

Deterioration of DDT Applied as a Residual Spray

The effect of factors such as temperature, ultra-violet light, flaking, cleaning of surface, and humidity on deterioration of DDT has been previously reported in "Summary of Activities" No. 6, 7, and 8. The following section on the effect of fly activity in causing loss of DDT concludes this investigation of factors causing deterioration of DDT. All techniques employed have previously been reported.

Effect of Fly Activity in Causing Loss of DDT Residual

The effect of fly (Musca domestica) activity in causing ineffectiveness of DDT was tested to determine if flies had actually removed the DDT or whether the DDT was masked. Panels of glass, paper, and wood surfaces were sprayed with 5 percent DDT in kerosene and 5 percent DDT in emulsion at the rate of 50 milligrams of DDT per panel. A standardized technique was used to test the flies against the treated surfaces. The possibility that flies remove DDT was tested and reported previously, but is summarized in the following paragraph to give a better picture of the effect of flies on DDT.

In order to determine if flies remove DDT from treated surfaces, two series of identical type panels were prepared. Series A, during a four-month period of time, was given twenty 60-minute exposures to approximately 100 flies. The toxicity of each surface at the start was very good, but the toxicity at the end of the four months was very poor, except on emulsion-wood type panels. Series B was handled in exactly the same manner as series A, except that no flies were exposed to series B. Panels similar to series B were highly toxic after six months. At the end of four months, the DDT was removed from all panels in both series. The difference in amount of DDT on corresponding panels in series A and B was not significant, except on the kerosene-glass panels. The amount of DDT which was recovered from each type panel was sufficient to have given high toxicity if the same amount of DDT had been freshly applied. These results showed that flies did not remove any significant amount of DDT, and that the amount of DDT

which was left should have been highly toxic, if the DDT was available for contact by the flies.

The above results indicated that DDT might be masked in some way. Three series of identical type panels were prepared. Series C was left uncovered in the laboratory for six months so that dust and dirt would settle on each panel. Series D and E, which were merely duplicates, were covered with a heavy grade of paper in such a manner that the covers were about 1/2 inch from the surface of the panels, and the covers were completely sealed along the edges and back with scotch tape. These two series, which were relatively free from dust, were placed in a laboratory desk for six months. At the end of six months, the panels in series C, D, and E were tested for toxicity to flies. Triplicate 15-minute exposure periods showed no significant difference in toxicity between corresponding panels in series C, D, and E. The twenty-four hour mortality was essentially 100 percent for all types of panels except the emulsion-glass and the kerosene-paper. The DDT was removed from each panel and the amount of DDT on corresponding panels in series C, D, and E was not significantly different. These results showed that DDT was not masked sufficiently by dust, dirt, or chemical means in the laboratory to cause low toxicity, but rather that the flies caused masking of DDT, possibly by defecation, regurgitation, etc. However, it has been previously shown that grease from kitchens causes decreased effectiveness.

These results, which are summarized in table 13, show that flies do not remove any significant amount of DDT from treated surfaces, but that flies themselves cause masking of DDT.

Table 13. Effect of fly (Musca domestica) activity in causing loss of effectiveness of DDT when applied at the rate of 200 mg. per square foot.

Description of Tests	5% DDT in Kerosene						5% DDT Emulsion					
	Glass		Paper		Wood		Glass		Paper		Wood	
	Recovery of DDT	Mortality	Recovery of DDT	Mortality	Recovery of DDT	Mortality	Recovery of DDT	Mortality	Recovery of DDT	Mortality	Recovery of DDT	Mortality
1. Series A. Twenty 60-min. exposures of approximately 100 flies. Analyses at the end of four months.	16%		96%		60%		79%		93%		91%	
		1%		1%		26%		1%		0%		82%
2. Series B. Followed identical procedure of Series A, except there were no flies used.	52%		93%		70%		81%		97%		85%	
		--		--		--		--		--		--
3. Series C. Panels were left uncovered in the laboratory for six months so that dust and dirt would settle on each one.	45%		76%		78%		68%		83%		82%	
		100%		84%		100%		37%		99%		99%
4. Series D. Panels were covered with paper for six months and were relatively clean.	55%		81%		63%		74%		83%		85%	
		97%		61%		100%		35%		98%		100%
5. Series E. Duplicate of series D.	52%		76%		91%		87%		83%		77%	
		98%		50%		99%		35%		100%		100%

Notes: 1. Mortality figures refer to the 24-hour mortality of females after a 15-minute exposure period.

2. Percentage recovery of DDT is based upon the amount of DDT theoretically applied.

Summary

Some factors causing the deterioration of DDT applied at the rate of 200 milligrams per square foot from either a kerosene solution or an emulsion containing 5 percent of DDT, has been ascertained on glass, paper, and wood surfaces.

Of the factors investigated, temperatures of about 140° Fahrenheit caused the greatest loss of DDT by both volatilization and decomposition of the applied DDT.

Although house flies (Musca domestica) did not remove DDT in significant amounts, the flies rendered residual deposits ineffective. DDT was not masked sufficiently under laboratory conditions by dust, dirt, or chemical means in six months to cause low toxicity to flies, but DDT was probably masked by defecation, regurgitation, etc., of flies.

Such factors as ultra-violet light, flaking, limited cleaning of most surfaces, and humidity are indicated by these tests to be of minor importance in causing deterioration of residual deposits of DDT.

RODENT AND ECTOPARASITE CONTROL BRANCH

C. M. Tarzwell, P. A. Woke, T. B. Gaines, J. G. McWilliams

Laboratory Insecticide and Acaricide Investigations

The search for a satisfactory acaricide has been continued. Tests have been made with several new materials in the form of dusts to determine their value for the control of the tropical rat mite Liponyssus bacoti. Results from laboratory tests with DDT indicate that this material does not appear to have practical value for the control of mature nymphs and adults of this species. Tests were made by directly exposing the various stages of the mite to pure DDT and to mixtures of 10 or 20 percent DDT in pyrophyllite for periods of 30 minutes to 2 hours. Test surfaces were covered with 200 or 500 milligrams of the material per square foot. Net kills at 24, 48 and 72 hours after exposure were generally under 25 percent. The occasionaly higher kills obtained, probably resulted from particularly susceptible mites. Since DDT is very effective against fleas, it might be desirable to combine it with a better acaricide for the control of rodent ectoparasites.

Sulphur: Sulphur has shown some promise for the control of the tropical rat mite. Kills of larvae, mature nymphs and adults after 30 minute exposures to sulphur dusted at the rate of 500 milligrams per square foot have been as high as 97 percent. Results of laboratory tests with sulphur are summarized in table 14. These results justify further studies with sulphur at various concentrations.

Table 14. Net kills of Liponyssus bacoti secured by 30-minute exposure to surfaces dusted at the rate of 500 mg. of sulphur per square foot.

Stage of Mites	Number of Tests	Average Effective Kills at Stated Intervals		
		24 hours	48 hours	72 hours
Larvae	3	48%	--	97%
Mature Nymphs and Adults	4	61%	70%	93%

Benzene hexachloride: Continued tests with benzene hexachloride (hexachlorocyclohexane) using mature nymphs and adult mites have given varying results. This variation appears to be closely related to temperature and the conditions of exposure indicating that kills may be largely due to a fumigant action.

Anthracene: Anthracene was combined with pyrophyllite to form a dust mixture containing 20 percent of the former. This mixture was ground for 2 hours in a ball mill to secure a suitable dust. Tests with this dust using the standard technique resulted in net kills of 56 and 83 percent at 24 and 48 hours, respectively, after exposure.

Hydroxymethyl flavan: A dust consisting of 75 percent celite and 25 percent hydroxymethyl flavan was tested against mature nymphs of the tropical rat mite. Net kills at 24 and 48 hours after exposure were 52 and 84 percent respectively.

Carbolineum: Carbolineum, a wood preservative, commonly used as an acaricide in poultry houses, appears to retain its toxic qualities for some time. Plywood panels painted with this material have continued to kill larvae, nymphs, and adult mites over a 5-week period. At the fifth week a two-hour exposure resulted in a net kill of 92 percent after 24 hours and 100 percent 48 hours after exposure. In this test, 188 mites were used on the treated panel and 216 on the untreated or check panel.

Other Materials: Ten additional substances were given preliminary tests as possible acaricides. It has been reported that, when sprayed on cloth, paper or wood, some of these have proved satisfactory against trombiculid larvae; however, when impregnated into clothing apparently not all were retained in sufficient amount during laundering to have practical use.

The dosage in exploratory trials at this laboratory was not regulated and five larvae were used in each test. The mites were released into and confined to 4-inch-square samples of the cloth or paper which had been saturated or liberally swabbed with the candidate acaricide, and the excess allowed to dissipate. The list of substances tested and the results are presented in table 15.

Table 15. Summary of exposure times required to produce 100 percent kill of tropical rat mites one to eight days after application of acaricide to test surfaces.

Substance Tested	Time required for 100 percent kill on each surface			
	Age of Treatment (Days)	On Treated Kraft Paper	Age of Treatment (Days)	On Treated Cloth
N-butyl phthalate	1	32 minutes	7	61 minutes
Benzyl Benzoate	1	33 minutes	7	55 minutes
Neo-neutracene	1	38 minutes	7	51 minutes
Bocene	1	33 minutes	7	49 minutes
Butyl acetate	1	40 minutes	7	49 minutes
Pyridine	2	No effect after 75 minutes	7	No effect after 77 min.
Di-methylphthalate	2	55 minutes	8	90 minutes
Indalone	2	23 minutes	8	65 minutes
Rutgers 612	2	56 minutes	8	50 minutes
Mixture of last 3	2	52 minutes	8	45 minutes

Field Insecticide and Acaricide Investigations

Tests on the Feasibility of Using Red Dye in DDT Formulations for the Control of Rat Fleas

Field and laboratory tests have been conducted on the feasibility of using a red dye as a coloring agent to suggest the poisonous character of DDT-pyrophyllite formulations used for the control of rat fleas. The material under consideration, consisting of 0.2 percent red dye added to a formulation containing 10 percent DDT and 90 percent pyrophyllite, was submitted for testing by Lucky Heart Laboratories of Memphis, Tennessee. It has been tested for effects on rats, effects on the toxicity of DDT to rat fleas, and coloring effect on wet surfaces. A sample of the red dye which was also submitted has been tested for effects on rats and coloring effect on wet surfaces.

Effects on Rat Behavior: In order to detect any avoiding action of rats due to the red dye, a Norway rat was placed in a 1 x 2 foot cage, the solid bottom of which had been covered to a depth of approximately one-eighth inch with the red dye. Another rat was placed in a similar cage, half the floor of which had been covered with a formulation containing 10 percent DDT and 90 percent pyrophyllite. Both rats stayed in the dusted portions of the cages for practically all of a six-hour period of observation.

In addition, the farm buildings which were dusted for rat-flea control with the 10-percent-DDT dust containing 0.2 percent of the red dye were checked the following morning to determine whether or not the rats seemed to avoid the colored dust. Tracks were found in most of the patches

of dust and the dust around the mouth of some of the burrows had been almost completely wiped up.

Effects on the Toxicity of DDT: Laboratory and field tests were conducted to determine the effect of the red dye on the toxic qualities of DDT. For the chemical studies, five samples were taken from different parts of the 10-percent-DDT dust containing 0.2 percent of the red dye. Tests showed an average DDT content of 9.4 percent. This does not necessarily mean that the red dye has adversely affected the DDT content, since it has been found on other occasions that commercial 10-percent-DDT dust products vary as to the actual DDT content.

Studies were also conducted to determine the toxicity of the same material to rat fleas in the laboratory. One adult Norway rat was dusted lightly with the 10-percent-DDT dust containing 0.2 percent of the dye and placed in a jar for comparison with another Norway rat which was placed in a similar container but was not dusted. Fifteen Xenopsylla cheopis were placed on each rat. After a 24-hour period, both rats were killed and the fleas recovered. Fourteen dead and no live fleas were recovered from the dusted rat. Thirteen live and two dead fleas were recovered from the check rat.

Field tests for determining any adverse effects of the red dye on the toxic qualities of DDT were conducted at a rat-flea-infested farm. The standard equipment for applying DDT dust for rat-flea control was used in applying 14 pounds of the dust containing 0.2 percent of the red dye to a feed barn, horse stable, and chicken house. The dust was applied on

January 30, 1947 after pretreatment studies of the rat-flea index had been made. Posttreatment studies were made 5 to 14 days after treatment. There was a range in the temperature during the trapping period from 75° F. before treatment to 24° F. after treatment. However, all trapping was done on days on which the minimum temperature was above 32° F. Suitable check stations were not available for comparing the rat-flea populations in treated and untreated areas. In the treated area, six rats trapped just before treatment bore a total of 124 fleas. After treatment five rats were trapped, none of which bore any fleas. Thus, an apparent control of 100 percent was obtained.

Coloring Effect on Wet Surfaces: A patch of 10-percent-DDT dust containing 0.2 percent red dye and a patch of the pure red dye were placed on a piece of stained pine flooring and on an unpainted piece of half-inch plywood. These materials were kept wet for two days after which time they were allowed to dry. When dry, an attempt was made to remove the patches by wiping them with a dry cloth and then with soap and water. On each piece of wood a light pink tint was left by the DDT dust containing 0.2 percent of the red dye. A dark red color was left on both pieces of wood by the undiluted red dye. The red dye is somewhat soluble in water and presents a definite staining hazard.

Conclusions and Recommendations: The Lucky Heart red dye when added to a 10-percent-DDT dust does not repel rats or decrease the toxic qualities of the DDT. However, it is somewhat soluble in water and will stain woodwork. Because of this, it cannot be recommended for widespread use in

10-percent-DDT dusts, since in many establishments and homes water is allowed to come in contact with the DDT dusts applied for rat flea control. The extent of staining is such that it might be objectionable to the occupants of many homes and establishments.

Laboratory Rodenticide Studies

Rodent Population Studies

A rat feeding station developed in England known as the "Elton Feeding Station" and used by the Bureau of Animal Population, Oxford University, has been given preliminary testing to determine the feasibility of its use in determining rat populations in establishments under conditions in this country. Through the use of such a method, if proved satisfactory, rat populations may be determined for field evaluation of rodenticides. The feeding station is a box made of wood with a small opening through which the rat may enter to feed.

Four of these feeding stations were placed in a small rat-proofed building containing 39 Norway rats. In developing this method for the estimation of rat populations, the first step was the determination of the normal average food consumption of the various species and sizes of rats over a 24-hour period. Growing-chick scratch feed was used in the feed stations over a period of 15 days. While the rats were becoming accustomed to these stations, the amount of food taken increased daily for a time and then leveled off at their average daily consumption, as indicated in table 16. The fluctuations on and after February 10 were

Table 16. Average amount of chicken scratch feed consumed daily by 39 Norway rats fed in Elton Feeding Stations placed in a ratproofed building.

Date	Amount of feed put out	Amount of feed recovered after 24 hrs.	Amount of feed consumed	Average feed consumption per rat	Remarks
2/ 4/47	5880 gms.	5354 gms.	426 gms.	10.92 gms.	A few grains spilled.
2/ 5/47	5354 gms.	4777 gms.	577 gms.	14.53 gms.	None
2/ 6/47	4777 gms.	4140 gms.	637 gms.	16.33 gms	None
2/ 7/47	4140 gms.	3369 gms.	771 gms.	19.79 gms.	None
2/ 8/47	3369 gms.	2602 gms.	767 gms.	19.66 gms.	None
2/ 9/47	2602 gms.	1858 gms.	744 gms.	19.07 gms.	None
2/10/47	5880 gms.	5212 gms.	668 gms.	17.12 gms.	Disturbance due to work done in building
2/11/47	5212 gms.	4576 gms.	636 gms.	16.31 gms.	"
2/12/47	4576 gms.	3832 gms.	744 gms.	19.07 gms.	None
2/13/47	3832 gms.	3176 gms.	656 gms.	16.66 gms.	None
2/14,15, 16/47	3176 gms.	Amt. in 72hrs 948 gms.	Gen. Ave. 3 days 742 gms.	19.02 gms.	Averages were taken over weekend
2/17/47	3888 gms.	3128 gms.	760 gms.	19.49 gms.	None
2/18/47	3128 gms.	2372 gms.	756 gms.	19.36 gms.	None

due to work being carried on in the rat house. It will be noted that the average amount of food consumed per rat was about 19 gms. after they became accustomed to feeding in the boxes. The average weight of the rats used in this experiment was 169 gms.

Food Preference Studies

If poison baits are to be used in poisoning operations, it is essential to have a suitable bait material. Four types of baits have been tested to date. Each of the bait materials were offered to the rats in the ratproofed building in separate feeding stations. The feeding stations were rotated in the building in order to keep the rats from continually eating one type of food because of being accustomed to feeding in one part of the building. The results are shown in table 17.

Table 17. Respective amounts of each of the four baits consumed during the feeding period.

Date Offered	Date Recovered	Amount of Bait Material Consumed			
		Wheat	Chicken Scratch	Rat Checkers Meal	Chicken Growing Mash
2/26/47	3/10/47	1627 gm.	3692 gm.	923 gm.	109 gm.

These results indicate in this instance at least that wet mashes are not very acceptable and that the chicken scratch feed is the most acceptable. This may be due to the fact that the rats had become accustomed to this food. In England wheat was found to be the best bait. Further studies of this type are needed.

Tests on "1080"[5/]

Work was continued on the tests with "1080"[5/] at reduced dosages. At present the concentration being tested is 4 grams of "1080" per gallon of water. This concentration has killed over half of the test rats in the first 24-hour period following its presentation.

Some study was devoted toward finding a satisfactory coloring material for use in "1080." This was judged to be necessary due to the danger of children drinking the solution. Colors were tested which were believed to be repulsive to children and not repellent to rats. Various dyes and colors were tried including vital stains and certified food colors. Greens, purples, browns, blues, and all the brighter colors were tried, but most of them appeared to be attractive to children as they resembled the soft drinks now on the market or other popular drinks such as iced tea, coffee, etc. These bright colors were discarded in favor of black or dark blue. Tests were made using India ink and blue-black writing ink to determine if water colored with these materials would be taken by rats as readily as pure water. The India ink was used at a concentration of 3.5 ml. per gallon of water and the writing ink at 40 ml. per gallon. Ten rats were used in the tests over a period of ten days. In carrying out the tests, two drinking tubes were hung in each of five cages, one contained the India ink mixture and the other water. The other five cages were set up in a similar way with the blue-black writing ink. Fifty ml. of the test materials were placed in the drinking tubes each morning and 24-hour checks were made to determine the amount taken.

5/ "1080" is a technical grade of sodium fluoroacetate manufactured by Monsanto Chemical Co., St. Louis, Mo.

During the ten-day period of observation, the following results were recorded:

Total amount of India ink and water taken.....1094 cc

Amount of plain water taken in cages where India ink was used....1237 cc

Total amount of blue-black ink and water taken.....1341 cc

Amount of water taken in cages where blue-black ink was offered..1382 cc

These findings indicate that the average amount of India ink taken per rat per day was 20.9 cc, while the plain water in the same cage was consumed at the rate of 24.7 cc per rat. There was no outstanding difference in the average amounts of blue-black ink and water taken in the other cages.

Preliminary work was undertaken on the development of bait materials. In some of these, agar-agar was used as a base. Peanut butter and oatmeal were added to the dissolved agar-agar to serve as an attractant as well as some coloring materials such as India ink or blue-black ink. After this mixture solidified, the consensus of laboratory personnel was that the colored cubes of agar-agar might be more attractive to children than the colorless ones. Baits prepared in this way were placed in ten cages, two cubes to a cage to test their acceptability to rats. All bait was eaten during the first night except the plain agar cube which had no attractant added to it. Other food was available in the cage while these baits were tested. These studies will be continued with "1080" added to the mixture.

Ratproofing Investigations

Tests to determine the relative resistance of new building materials to damage by rats were undertaken early in 1946. The main purpose of these studies was to determine the resistance to gnawing provided by fabricated composition materials used in ship construction. However, many of the materials tested may also be used for the construction or ratproofing of buildings and the findings will thus have application in general rat control programs. The samples which have been tested or are being tested have been furnished by firms engaged in the manufacture of building materials.

The method of testing now being used has been evolved from a variety of methods employed early in the study. Various types of cages, methods of exposure, and incentives were tried to induce the rats to gnaw the materials under test. The cage now used for confining the rats and the method of presenting the test panels are shown in figure 4. As indicated, the cage is 24" x 13 1/2" x 7" and is divided into two compartments by a 12" x 12" panel (A) which is inserted in a metal slot on each side of the cage and held firmly in place by a pinch brace. During periods when test panels are not in place, a plywood training panel with a hole in the lower left-hand corner is kept in place to train the rats to go through an opening into the front compartment (C) where food (F) and water (B) are kept. The nesting box (E) is kept in the larger compartment (D) where the rats are confined by the test panels. After training the rats to go through a small opening in order to secure food and water, the diet is somewhat

LEGEND:

A. Panel In Slot
B. Water
C. Food Compartment
D. Mat On This Side Of Panel During Test
E. Nesting Box
F. Food
G. Door

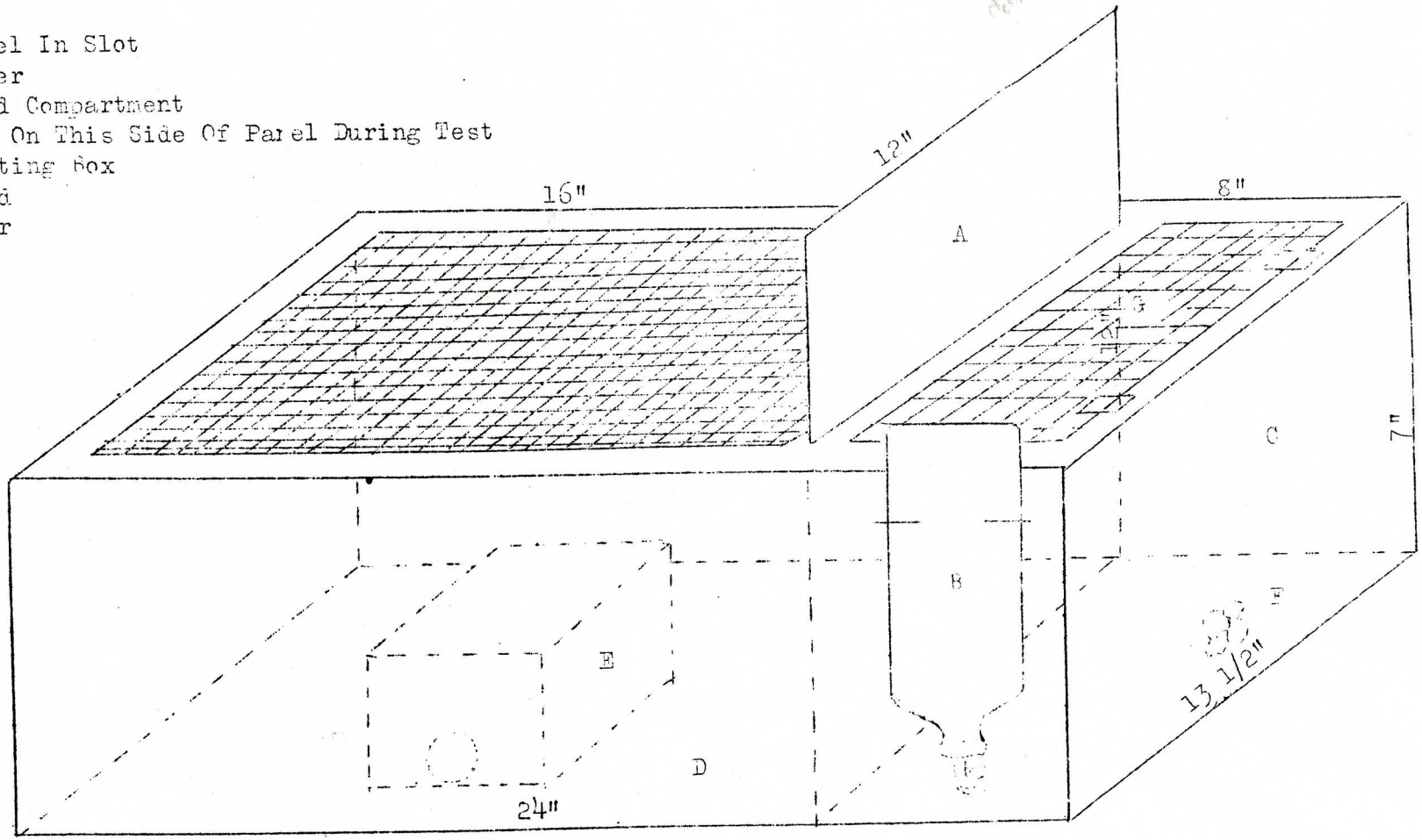

Figure 4. Panel test cages constructed of $\frac{1}{2}$" plywood completely lined on sides and ends with $\frac{1}{2}$" mesh hardware cloth. Top and door made of framed $\frac{1}{2}$" hardware cloth - bottom made of sheet metal - at no place is the wood left exposed to attack by rats.

reduced and a test panel is inserted each week-day night (Monday through Thursday) to determine if the rats are able to gnaw through the panel to gain access to food and water. Due to the fact that the rats are accustomed to going through the training panel at the lower left-hand corner, much of the gnawing effort is directed to that area. The cages have a metal bottom, an open top of 1/2 inch hardware cloth and a lining of the same material. As materials being tested are for use in ships, the ship rat Rattus rattus alexandrinus has been used in all tests. Because some rats are better gnawers than others, provision must be made for the exposure of all panels to all the rats. After each night of exposure, a panel is moved over to the adjoining cage for exposure that night and thus the panels are rotated through all cages being used in the tests. Test panels are inserted in the late afternoon and removed early each morning at which time any gnawing which took place during the night is recorded. The rats are given a limited diet during the Monday through Friday period, but they are given large feedings over the week-end.

To date, tests have been made with 24 different materials and tests are now underway with 25 additional materials. Some are tested to determine their value for ratproofing, while insulating materials are investigated to determine if they will be used as rat harborage. Early in the investigation, the panels were inserted flush with the bottom of the cage so no gnawing edge was presented. With the harder materials, it was impossible for the rat to begin to gnaw. In order to remedy this situation and to give the materials a more severe test, they were inserted so the

bottom edge was 1/8 inch from the bottom of the cage. Later this opening was increased to 1/4 inch as it was felt that even with the best construction there would be joints or openings of 1/4 inch. In several instances, tests were also made with the panel flush to the bottom but with a 11/16 inch hole drilled through the lower left-hand cover. Many of the panels were tested under each of the above 4 conditions.

Results:

Sample RP. #1 C-5 Marine board, 3/16 inch thick, Keasbey and Mattison Company, Ambler, Pennsylvania:

This material is very hard. It was exposed 4 nights flush to the bottom and 3 nights raised 1/8 inch from the bottom of the cage with no damage. Fifty nights of exposure when raised 1/4 inch resulted in some teeth grooves along the lower edge. A panel having a 11/16 inch hole bored about 1 1/2 inches from each edge was exposed for 18 nights with slight damage. There were grooves all around the opening to a depth of about 1/16 inch. Although few materials are entirely ratproof, it is apparent that this material is very hard and under ordinary conditions would be practically ratproof. Thirteen nights exposure of an additional panel flush with the bottom resulted in no damage. This material is fairly brittle and will break under a sharp impact.

Sample RP. #2 C-5 Marine board, 1/4 inch thick, Keasbey and Mattison Company:

This panel is similar to #1, apparently differing only in being thicker. Four nights exposure flush and 3 nights raised 1/8 inch from the bottom of

the cage resulted in no damage. There were only slight scratches on the bottom edge of the panel after 53 nights exposure while raised 1/4 inch from the bottom. A second panel exposed flush with the bottom of the cage for 14 nights showed only a discoloration along the lower edge, and 20 nights exposure with a 11/16 inch hole drilled through the lower corner about 1 1/2 inches from each edge resulted in only a few grooves around the circumference of the opening. This material will break under a sharp jar or impact.

Sample RP. #3 C-4 Marine board, 3/16 inch thick:

Exposure for 4 nights flush with the bottom and for 3 nights 1/8 inch from the bottom of the cage resulted in no damage. When set 1/4 inch from the bottom of the cage, 52 nights exposure resulted in some damage. Grooves were made all along the lower edge, some of which were 1/8 inch deep. Given sufficient time and ideal conditions, it is probable that rats would go through this material. If a gnawing edge is not present, however, it is doubtful if rats would penetrate, since 15 nights exposure with the panel flush on the bottom resulted in no damage. Twenty nights exposure of the panel with a 11/16 inch hole resulted in extensive gnawing and a slight enlargement of the hole. In time the rats would have gone through this opening. This material is resistant to blows or sharp jars and is better in this respect than sample 2.

Sample RP. #4 C-4 Marine board, 1/8 inch thick, Keasbey and Mattison Company:

Four nights exposure flush to the bottom of the cage and 3 nights raised 1/8 inch from the bottom caused no damage, but when raised 1/4 inch

from the bottom there was considerable gnawing along the lower edge during 51 nights. The teeth grooves were over half the thickness of the panel, but the edge had been gnawed back only about 1/8 inch. Fifteen nights exposure to gnawing on another similar panel set flush on the bottom resulted in only a discoloration of the lower edge. A 11/16 inch hole in one corner permitted considerable gnawing and the enlargement of the hole to over 1 inch across in 19 nights. Exposure for a larger period would have allowed large rats to penetrate this opening as a small rat went through on the 19th night. This material is resistant to blows or jars.

Sample RP. #5 C-3 Marine board Finalite finish - 3/4 inch thick. Keasbey and Mattison Company:

Six nights exposure when flush with the bottom of the cage resulted in only slight gnawing along the lower edge. When raised 1/4 inch from the bottom the rats gnawed on the panel 16 nights out of 26 and went through the panel on the 26th night of exposure. A similar panel placed flush with the bottom for 34 nights was not penetrated, but it was very extensively gnawed and with further exposure would have been penetrated. This material is soft, relatively easily gnawed and is not ratproof regardless of instillation.

Sample RP. #6 Standard Asbestos Lumber:

Laminated construction, fire resistant, smooth surface, gray color, 1/4 inch thick, strong and dense.

This material is very similar to panel No. 1. Four nights exposure flush to the bottom of the cage and 2 nights raised 1/8 inch from the

bottom caused no notable effects. During a period of 46 nights with the panel raised 1/4 inch from the bottom there was some grooving and pitting of the bottom edge. The deepest penetration was about 1/16 inch. Another panel which was exposed for 13 nights flush with the bottom received only a few scratches and nicks along the lower edge. A 11/16 inch circular hole drilled through the lower right-hand corner of the panel 1 1/2 inch from each edge was subjected to gnawing for 19 nights. There was some grooving all around the hole and chipping in a 1/4 inch area on one side over half way through the panel. The hole was not enlarged and a great deal of time would be required for the rats to gnaw their way through even under these ideal conditions. When properly installed this material could be considered as being relatively ratproof. It is tough, hard and resistant to blows or jars.

Sample RP. #7 Century APAC Board, 3/8 inch thick and made of asbestos fiber and Portland cement, Keasbey and Mattison Company:

No damage resulted from tests over a period of 18 nights with the panel flush on the bottom of the cage or 4 nights with the panel 1/4 inch from the bottom. Only a few grooves were made around a 11/16 inch circular hole during 14 nights. This material is similar to panels No. 1 and 6 and can be considered relatively ratproof when carefully installed. However, it will break under a sharp blow or jar.

Sample RP #8 Composition board with plaster surface backed with metal lath and 1 inch of asbestos fiber held in place by a metal sheet at the back. Keasbey and Mattison Company:

The surfacing of this panel was removed along the lower edge during 6 nightly tests even though the panel was flush with the bottom of the cage. Although the material is relatively ratproof due to the metal lath and the sheet metal back, it would be subject to extensive rat damage on the surface. Further, it would be difficult to install the panels so there are no openings between the sections of metal lath when the surface covering is removed. Such openings would expose the insulating material which is readily attacked and removed by the rats even though it is only 1 inch thick.

Sample RP. #9 Marine sheathing, plain fishish, 3/8 inch thick. Johns Mansville Company:

When exposed to gnawing, this panel was not attacked by the rats during the first 4 nights; but during the next three nights, the rats gnawed through it to gain access to the food and water. Since this panel was flush to the bottom, it cannot be considered ratproof. It offers little resistance to gnawing. This material is fairly resistant to sharp blows or jars.

Sample RP. #10 Marine Sheathing, plain 1/2 inch - Johns Mansville Company:

When placed flush on the bottom of the cage, this material was not attacked during 5 nights; but when raised 1/4 inch from the bottom, it was attacked the first night, and the rats went through it on the 19th night. When a notch two inches long and 1/8 inch deep was cut in the lower edge of a panel of this material, it was attacked at once by the rats and was considerably damaged during 26 nights. With more time the panel would

have been penetrated. A second panel which was set flush with the bottom of the cage for 31 days was only slightly damaged.

If this material is set so all joints are tight, it will be resistant to gnawing; but if there are openings of 1/8 to 1/4 inch, the rats will penetrate it in a few weeks. This material is quite resistant to sharp blows or jars.

Sample RP. #11 Marinite, plain finish, 3/4 inch thick. Johns Mansville Company:

This material is much lighter than samples 9 and 10, and it has a hard glazed surface. However, its inner core appears to be soften than the materials in samples 9 and 10. When exposed flush with the bottom of the cage for 30 nights, the surface layer was broken; and the rats gnawed to a depth of about 1/4 inch. It is probable that they would penetrate this material if given sufficient time. However, if the edges of this material are protected, the surface is so hard and smooth rats would not be able to gnaw it. This material is resistant to blows or jars and is far superior to sample 12 in this respect.

Sample RP. #12 Marinite, standard base finish, 3/4 inch thick, Jones Mansville Company:

This panel is similar to number 11, but it lacks the hard glazed surface. Thirty-five nights exposure flush with the bottom of the cage resulted in only light damage along the lower margin. While this material is resistant to gnawing, it is not ratproof and cannot compare to samples 1, 2, 3, 6 or 7. This material breaks readily with a jar or sharp blow.

Sample RP. #13 Marinite, Marine Veneer on both faces, 7/8 inch thick, Johns Mansville Company:

The central core of this material is similar to that in samples 11 and 12. However, it is faced on each side with a very hard glazed material almost 1/8 inch thick. This surface material is resistant to gnawing and was only slightly roughened along the lower edge by 20 nights exposure flush with the bottom. A circular hole 11/16 inch in diameter was bored through a second panel which was exposed for 20 nights. The surface facing was nicked around this hole, but it was not penetrated. This material can be considered to be relatively ratproof. It compares fairly well with samples 1, 2, 3, 6, and 7. It is resistant to blows or jars.

Sample RP. #14 Transite, asbestos wood, 1/4 inch thick. Johns Mansville Company:

This material was left flush with the bottom of the test cage for 27 nights with no change other than a roughening and discolorization of the lower edge. Another panel having a 11/16 inch hole bored in the lower right-hand corner was exposed for 29 nights. During this period the rats tried constantly to enlarge the hole and succeeded in making a band of nicks around the hole and a deep notch on one side. The hole was not enlarged, but the notch was extended almost through the panel and over a long period of time with ideal conditions, rats would probably penetrate this material. However, it is very rat resistant and under conditions of careful construction, it would probably be ratproof. It is resistant to blows or jars.

Sample RP. #15 Marine veneer, 3/16 inch thick, Johns Mansville Company:

This is a hard material very similar to sample #14. Twenty-eight nights exposure flush with the bottom of the cage resulted in only a slight roughening of the lower edge of the panel. With proper construction so there are no openings and edges are protected, this material would be relatively ratproof. Further, it is very tough and resistant to jars and will readily withstand sharp blows with a hammer.

Sample RP. #16 Marine veneer, 1/8 inch thick, Johns Mansville Company:

This is a tough material which is resistant to sharp blows or jars. It is not as hard as sample 15, but it compares fairly well with it. Twenty-seven nights exposure flush with the bottom of the cage resulted in only a slight nicking of the lower edge. It is, therefore, resistant to rat attack. Further studies will be made with this panel raised 1/4 inch from the bottom of the cage.

Sample RP. #17 Transite Asbestos Wood, 1/2 inch thick, Johns Mansville Company:

This sample is a heavy, hard, dense, strong material which is resistant to sharp blows or jars. It would require a hard blow to dent it and it is not liable to scratch. Seventeen nights exposure flush with the bottom of the cage resulted in only a slight roughening of the lower edge. Another panel having a 11/16 inch diameter hole bored through one corner about 1 1/2 inches from the edges was exposed to rat gnawing for

19 nights. The rats were unable to gnaw this material and succeeded in only scratching the surface around the hole. This material may be considered as relatively ratproof.

Sample RP. #18 Perforated marine veneer. Sound proofing board with 5/32 inch holes 1/2 inch apart. Board 3/16 inch thick. Johns Mansville Company:

A sharp blow will break this board if it is unsupported due to the fact that it is weakened by the holes. The material is quite tough and strong, however, and is resistant to jars. It was exposed for 43 nights with its lower edge flush on the bottom of the cage. Most of the holes in the lower 3 inches of the panel had been gnawed around the edges, but none had been gnawed sufficiently to actually enlarge the holes. Active gnawing extended to a height of 4 1/2 inches and there was some gnawing to a height of 7 1/2 inches. This material would be penetrated if exposed to rats over long periods, but it does offer considerable resistance to gnawing.

Sample RP. #19 RC#1 Pressed cork with composition face dented in the center, Johns Mansville Company:

This panel is composed of compressed cork 6 inches thick having a two-ply facing of hard material over 1/2 inch thick. This facing is dense, hard and strong and is resistant to blows or bruises and will withstand sharp jars fairly well. The face of this panel was damaged to determine if the rats would penetrate the damaged area. During a period of 13 nights, wild Norway rats did not attack the damaged area. The panel was

then reversed to expose the insulating material. The rats burrowed into this at once. Over a period of a few nights, they made extensive excavations in the material and built a nest which they occupied for over 2 months. Mites became numerous in the nest and throughout the insulating material.

Although the insulating material will be used as harborage, the facing is relatively ratproof and under proper construction will probably prevent rats from reaching the insulating material. The backs must also be protected with a similar facing or with metal.

Sample RP. #20 #2Z. Brown fiber type insulation, 6 inches thick, with a 2-ply, hard cement and fiber facing 1/2 inch thick, facing dented in the shape of an equilateral triangle 2 1/2 inches on a side - manufactured by Johns Mansville Company:

During 29 nights exposure the facing was not attacked along the edges or at the injured area. On August 15, the facing was removed to expose the insulation. The rats burrowed into it and lived in it until November 5 when the test was discontinued.

Sample RP. #21 #9RC Pressed cork insulation with composition 2-ply face. Johns Mansville Company:

The face of this panel was damaged severely prior to testing. During 35 nights exposure, the rats failed to attack the panel facing along the edges or in the injured area, even though good gnawing edges were provided. When the facing was removed on August 15 to expose the insulating material, they readily burrowed into it and formed a nest. Six young were born on September 22, and the rats remained in the material until November 5 when the study was discontinued.

The facing material is apparently rat resistant and affords protection to the insulating material.

Sample RP. #22 8-Z and 8ZA. Brown fibrous insulation with a composition face (2-ply) about 1/2 inch thick. Johns Mansville Company:

The face of this material was dented by a heavy blow prior to testing. During 17 nights exposure the rats failed to attack the panel at any place. When the facing was removed August 15, they burrowed into the insulating material and nested. Young were born in October and the rats remained in the material until November when the study was discontinued. The facing material is rat resistant and provides protection for the insulating material.

Sample RP. #23 #1B. Soft, brownish, fibrous material faced with cement composition board 2-ply 1/2 inch thick. Insulation 6 inches thick. Johns Mansville Company:

The surface of this panel was damaged to test if rats would penetrate to the insulation through a damaged area. Neither the edges nor the damaged area of the facing was attacked by the rats during 31 nights exposure. When the facing was removed the insulation was utilized as harborage by the rats. The rats remained in the insulation from August 15 until the test was discontinued in November.

Conclusions

Very few materials can be considered absolutely ratproof. When rats are abundant and hungry, they will penetrate even concrete if given sufficient time and suitable conditions. They have been known to penetrate 4 inches of concrete under such conditions. Any composition material can

therefore at best be considered as only relatively ratproof.

Studies to date on the various materials tested indicate that samples 1, 2, 6, 15, and 17 are the most resistant to attack by rats and can be considered to be fairly ratproof. Samples 3, 7, and 16 are resistant and under conditions of careful installation should give protection from rat attack over considerable periods. Samples 4, 11, 12, and 18 are only moderately resistant, while samples 5, 8, 9, and 10 are unsatisfactory.

Samples 19, 20, 21, 22, and 23 were insulatory material 6 inches thick with a hard, 2-ply composition facing 1/2 inch thick. The facing of all these samples were very rat resistant and were not damaged in any of the tests even though they were injured or dented to give a gnawing edge.

Rats readily burrowed and nested in the insulating material of all these panels. They successfully produced young in two of them, and it is apparent that the insulation must be protected. It is believed the facing is adequate protection for one side, and the material could be used with safety if all other surfaces are equally well protected.

Compiled and Edited:

William M. Upholt

William M. Upholt Biologist

APPROVED: April 23, 1947

S. W. Simmons

S. W. Simmons, Sanitarian (R)
Chief
Technical Development Division

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EMORY UNIVERSITY FIELD STATION

REPORT OF ACTIVITIES

July 1, 1944 --- August 31, 1945

EMORY UNIVERSITY FIELD STATION

REPORT OF ACTIVITIES FOR THE PERIOD

JULY 1, 1944 - AUGUST 31, 1945

Operated in cooperation with

Office of Malaria Control in War Areas,
U. S. Public Health Service

and

Water Resources Branch,
U. S. Geological Survey

September 7, 1945

INTRODUCTION

The period covered by this report, July 1, 1944 through August 31, 1945, has been one of the most interesting and productive since the establishment of the Field Station. During this period investigations were expanded and redirected on the basis of information obtained through application of techniques and procedures developed at the Field Station. Additional investigations were instigated to determine efficiency of control procedures being conducted by other agencies, since the Field Station area provided an ideal place to conduct these observations because of the large amount of basic data already accumulated. This represents the partial achievement of one of our objectives to provide a facility where sufficient preliminary observations had been conducted to provide a foundation for related work. In this way it is possible for investigators to conduct various short term experiments and observations which would not be possible were the basic data not already available. The plausibility of the program has been confirmed by the interest and financial support of various agencies concerned with the type of work in progress at the Field Station. Expenditures by cooperating organizations exceeded the appropriation by the Research Committee for operation of the Field Station.

In May 1942 activities of the Field Station were curtailed to permit professional personnel to assume positions more directly connected with the war effort. An interim program was instigated to assure continuity of data during the period of curtailed activity. Subsequent to curtailment of activities much of the information collected at the Field Station was made available to agencies engaged in malaria control operations who thus became informed of the Field Station work. In appreciation of significance of the

Field Station program to the overall malaria problem the U. S. Public Health Service entered into a cooperative agreement with Emory University to permit resumption of full scale operations. Through this arrangement prerogatives of operation remain solely in the hands of the Field Station Research Committee. The Public Health Service agreed to provide personnel, various equipment and other services in support of the project. Through this arrangement facilities have been available to accomplish much work not possible heretofore. The greatest handicap has been lack of adequate laboratory space. This obstacle will be overcome with the completion of the new laboratories.

Additional outside support was obtained by a cooperative agreement with the U. S. Geological Survey through the State Department of Conservation, Division of Mines, Mining and Geology whereby a stipend to support the hydrological phases of the program was made by the Research Committee. This was matched by an equal amount of federal funds by the U. S. Geological Survey. Through this arrangement professional services were obtained which would have been otherwise unavailable. The interest of the State and Federal departments is very gratifying.

Since the establishment of the Field Station a policy has been followed to undertake investigations which can be done only or more advantageously in the Field Station area. The original proposal outlined a program of investigation designed to explore the factors responsible for the occurrence of malaria. This represented the first attempt to conduct extensive field observations on the natural history of this disease. It was felt that the accumulation of this fundamental information would provide a basis for conducting many field observations on problems related to the epidemiology of the disease under natural conditions. Several years of work were anticipated before this information would be useful. The accumulation of these data for six years

indicate that hitherto unavailable information is being obtained and its usefulness and significance is best illustrated by the interest of other agencies in this program.

ACTIVITIES DURING THE PERIOD JULY 1, 1944 - AUGUST 31, 1945

The first consideration after resumption of full-scale operations was compilation and analysis of data collected during the period of curtailed activity (May 1942 - June 1944). For this period personnel was available only for collection of data. As a result information was available only on field record sheets. It was also necessary to modify collection procedures to permit accumulation of data in connection with additional work which was undertaken.

For convenience in reporting various activities the procedure used in previous reports is again employed and activities are grouped under (1) Epidemiological Investigations, (2) Biological Investigations and (3) Hydrological Investigations.

1. Epidemiological Investigations.

When work was begun at the Field Station in 1939 no information was available as to malaria rates or location of malaria cases and malaria foci. The first epidemiological procedure was to determine where malaria occurred. To accomplish this extensive surveys were made over all of Baker County and adjacent areas. A total of 6,215 blood smears were taken and examined for malaria. With this procedure it was established that by far the majority of the malaria occurred in the northwestern portion of Baker County. This area was selected for investigations and experimental work since it was necessary that an appreciable amount of malaria be available for study. Surveys were continued westward into Early County until an area of approximately 400 square miles had been thoroughly investigated for the occurrence of malaria. Efforts were intensified in this area during the next year (1940) resulting in establishment of a much higher malaria rate for this area, although

malaria was declining naturally over the region as a whole. From 3,840 blood examinations, 291 were positive for malaria, as compared with 99 positive smears from the 6,215 collected during the previous year. This apparent increase in rate was due to selective collection.

In this area selected for experimental work and observations, measurements of malaria were made periodically. In addition a group of about 1000 persons was selected for intensive study and were visited regularly by the nurses. An accurate record of malaria symptomatology was kept on each individual. Blood smears were made periodically and also upon the manifestation of any symptom indicative of malaria. This group has served to supply an index of the amount of malaria occurring in this section. Information is also obtained on the distribution of malaria in age groups and on the relation of clinical malaria to positive blood smears (parasitemia). A marked reduction in the malaria rate was noted in 1941. Only 43 positive smears were detected from 3,629 collected. In subsequent years this was further reduced with the result that 15 positive smears were detected from 2,292 examined in 1942 and in 1943 only 4 positives were obtained from 1,272 smears examined. In 1944 there was only 1 positive smear out of 3,088 blood smears examined. Not a single case has been detected to date in 1945. The number of persons with symptoms indicative of malaria is closely correlated with the number of positive blood smears, indicating that clinical malaria is detected by the nurses with a fairly high degree of efficiency. The following table indicates the number of examinations and the number of positive blood smears over a period of the last six years. In 1942 and 1943 fewer smears were collected in surveys because of reduction in personnel and a request from the State Health Department to submit as few smears as possible until pressure of their work was relieved.

SUMMARY OF MALARIA MORBIDITY

YEAR	MALARIA SURVEYS: NUMBER OF PERSONS EXAMINED IN SURVEYS	MALARIA SURVEYS: NUMBER OF POSITIVE SMEARS	CLINICAL MALARIA: NUMBER OF PERSONS WITH POSITIVE Symptoms	CLINICAL MALARIA: NUMBER OF POSITIVE SMEARS
1939	6002	47	213	52
1940	2368	24	1472	267
1941	3132	18	497	25
1942	2012	3	280	12
1943	996	1	276	3
1944	2948	0	140	1
1945*	86	0	17	0

* Through May.

The reduction in malaria morbidity has been observed in most other parts of the world where malaria occurs. No adequate explanation of this phenomenon has been offered. There is certainly no reason to believe that malaria will not return. It is a pleasant thought to assume that control measures, where applied, have been responsible for this reduction. From data collected in the Experimental Area where no control measures were applied, the same degree of reduction occurred.

One of the primary objectives was to investigate factors related to natural variations in malaria. At the time of instigating the program there was no way to anticipate such a drastic reduction in such a short time. It is not known whether malaria, during periods of remission, recedes to restricted foci where normal transmission occurs on a greatly reduced scale or if it is maintained at a subclinical level in the population, reappearing under suitable

conditions. Either of these conditions is entirely possible. The first possibility would probably depend upon physical condition, the second on the inherent characteristics of the biological material involved. In either case, observations at the Field Station are designed for the quick detection of cases when malaria reappears. It is possible that information on the method by which malaria is perpetuated during periods of reduced morbidity will be obtained.

In addition to experimental work, regular nursing visits are made to the persons on Ichauway Plantation. This requires virtually the full time of one nurse. During 1939 and 1940, prophylactic Atabrine was administered to the residents of Ichauway. This has not been done subsequently because of the negligible malaria rate.

In addition to work directly concerned with malaria epidemiology a great deal of general public health work was also done. Through May 1945 these activities included 2,035 immunizations for typhoid fever, 194 for diphtheria, 27 for smallpox; collection of 346 specimens for blood test, 72 specimens for hookworm, and 263 specimens for miscellaneous tests. Twenty-five persons were examined for tuberculosis and 136 tuberculin tests were done.

2. Biological Investigations.

Biological activities were designed to investigate characteristics and habits of the malaria vectors. During the period of curtailed activity no work on such problems was possible. It was possible only to maintain collection of qualitative information on breeding of Anopheles mosquitoes in the Experimental Area. Previous work at the Field Station had resulted in the development of methods for making measurements of mosquito populations. Continued observations on the value of these methods were made. In connection with attempts to measure Anopheles populations, considerable attention was given to the habits

and activity of adult mosquitoes. It is interesting that much more information is available in the literature on the larvae of mosquitoes than adults. This is due largely to the fact that control methods have been usually directed against the aquatic stages. The advent of DDT as one of the most promising insecticides for malaria-mosquito control necessitates rather comprehensive information concerning the adult stage before accurate appraisal of the efficiency of this method of malaria control can be made. Much of the data already available at the Field Station is directly applicable to this problem. And it provides an excellent basis for the projection of future work along these lines. This is discussed in greater detail subsequently.

Dispersion of Mosquitoes.

During the period covered by this report work was done on the dispersion of mosquitoes from breeding places and blood meal sources. This information is needed for the proper evaluation of the number of mosquitoes found in collecting places near ponds in which malaria-carrying mosquitoes breed and in places where they obtain blood meals. It is necessary to know if there is a cumulative effect or if the number found represents only the number which entered the shelter during the previous night. Investigation of the problem necessitates that mosquitoes be marked in such a way that they can be recognized when subsequently collected. The usual method employed necessitates collection of large numbers of mosquitoes and placing them in a screen cage where they are sprayed with a dilute solution of some dye. When collections of mosquitoes are later examined for marked individuals, the specimens are placed on a porcelain plate and a drop of solution which will dissolve the dye is placed on each mosquito. If the specimen has been previously dyed, the destaining solution

will acquire the color of the dye applied. Using this method several observations were made on groups of approximately 2,000 mosquitoes. The maximum number recovered was about 100 specimens or about 5%. To obtain reliable information it is obvious that a large number of experiments must be conducted.

Some interesting observations were made from these experiments. It was noted that dispersion from breeding places is fairly rapid. Only half as many marked mosquitoes are captured on the second day after release of the stained specimens as were taken the day after. The number decreases rapidly and after the third or fourth days only an occasional specimen is detected. It is significant however, that a few individuals are collected three or four weeks after release. The fact that any specimens are collected after the first day is interesting since it indicates an appreciable amount of daily activity. Daily collections are fairly consistent as to the number of specimens collected, indicating that there is not an appreciable accumulation of mosquitoes which emerge from a breeding place. The proportion of males collected near the breeding place after staining is greater than the percentage of females. This confirms the opinion that male mosquitoes are less likely than females to move voluntarily from the breeding place since there is no stimulus to seek blood meals. The length of life of male mosquitoes is believed to be comparitively short, lasting for only a few days. Male mosquitoes have been recovered, however, which were marked three weeks previously.

Studies on dispersal of Anopheles from resting places near sources of animal blood indicated that there was no tendency for the mosquitoes to remain in these shelters after the blood obtained was partially digested. The length of time which this required was not long enough to cause accumulation of specimens. In no case was there any indication of "homing instinct" or tendency of mosquitoes to return to the place where they were originally collected.

Studies on methods of marking and detection of marked mosquitoes.

Handling mosquitoes in the manner described above is very time consuming. In view of the desirability of conducting experiments in which marking and detection of marked individuals is necessary, studies were undertaken in an attempt to find a more convenient method. Most of the time required by the method used was devoted to examination of collections for the marked specimens, since each mosquito was necessarily handled separately. The possibility of marking mosquitoes by a fluorescent material which could be easily detected under an ultraviolet light was investigated. Compounds which produce blue, red, and green fluorescent colors have been successfully used. These are applied to the mosquitoes either in the form of a dust mixed with gum arabic, which cause the material to stick to the insect, or in solution. When collections are examined for marked specimens large numbers are passed under an ultraviolet light in a darkened room. The marked specimens are readily detected. This method has not yet been subjected to critical field tests but the results so far obtained are promising enough to leave no doubt that the method can be used under field conditions with much greater ease than methods previously employed.

Observations on Anopheles populations.

During the current season Anopheles densities were at lower levels than previously observed in the Field Station area. The factors which are responsible or related to this are not immediately apparent. It is hoped, however, that by the end of the season some related factors can be determined from analysis of the data being obtained by hydrological observations. If this is not possible, variations of factors may be detected when the population begins to increase. The extent to which the population has been reduced is almost unbelievable. In many instances mosquitoes have not been available in sufficient

animals for experimental work. Only a few dispersion studies could be done during 1949 because of the small number of specimens available.

The immediate effect of such reduction in *Anopheles* densities on malaria morbidity is obvious. This reduction could have been in progress for some time and methods for estimating *Anopheles* populations not sufficiently critical to detect changes which did not occur in the terminal phase of the regression. In other words, there may have been a gradual reduction of malaria vectors for the past few years when malaria cases were progressively fewer. Enough vectors may not have been present to effect malaria transmission but the methods of measuring density of vectors were not developed to the extent that such changes could be detected. In any event an upswing in *Anopheles* populations may be expected. Every effort is being made to measure changes in natural conditions which may be responsible for or related to this variation. It has been noted from information obtained from precipitin tests on mosquitoes which have had blood meals that a very low percentage of malaria vectors are feeding on human blood. By far the majority of specimens have fed on cows. This source of blood is available usually near the mosquito breeding place. It may be that the mosquito population is reduced to such a low level that the normal dispersion and nocturnal activity takes place in a much more restricted area than would be involved if the population were greater. When the number of mosquitoes increases, it will be interesting to see if the proportion of *Anopheles* feeding on humans also increases.

An attempt was made to collect large numbers of mosquitoes from dwellings, outbuildings, stables, barns, shelters near breeding areas, and other types of resting places to determine the rate of feeding on human blood of mosquitoes taken from shelters of these various types. The plan was that selected areas were to be sprayed with DDT to determine if the percentage of human feedings

was reduced in these areas as compared with the rates in untreated areas. As indicated above the number of mosquitoes collected was extremely low. In this instance the numbers obtained were so low that conducting this experiment was not practical as the results would be statistically insignificant.

Studies on the Winter Activity of Anopheles

As part of investigations on Anopheles biology a comprehensive study was made of the winter activity of the malaria vector. Knowledge concerning the over-wintering of Anopheles is very deficient. Since Anopheles populations undergo a drastic numerical reduction during the colder months, the possibility of applying control measures when the population is reduced is worthy of consideration. The overwintering places of Anopheles are not well known and the stage or stages of the insect which can survive the winter are uncertain. In colder latitudes enormous numbers of adult mosquitoes have been observed in hibernation during the winter. It has been commonly believed, however, that in the region including the Field Station area, there is no true hibernation but breeding occurs throughout the year on a greatly reduced scale. There has been little evidence to substantiate the belief. Previous efforts have been directed to attempt mosquito collections from the usual summer resting places. Such searches have not supplied much information.

During the last winter large numbers of Anopheles were collected from hollow trees which seems to be a principal cold weather resting place. The insects are not found in the easily accessible portion of the tree but seek the more sheltered recesses. The mosquitoes are collected by fumigation with sulphur dioxide which is generated by burning a thick paste made of sulphur and fuel oil in a composite burner. The heat generated causes the mosquitoes to become active. In a short time they are overcome by the gas and fall to

the base of the tree in which white cheese cloth has been spread previously to facilitate collection. The treatment does not kill the insects but renders them unconscious for several minutes, during which time they are transferred to large glass test tubes and stored in the refrigerator until examined. In this connection, it is interesting that mosquitoes survive for several days in the freezing compartment of the refrigerator.

Mosquitoes collected in the manner described were subsequently examined for the purpose of evaluating, 1. the external appearance to obtain an estimate of age based upon the condition of the wing scales, 2. degree of blood ingestion, 3. degree of ovarian development. Five categories were used for each item. Specimens were also classified as to the amount of fat present. This was done by estimating the per cent of space of the haemocoele occupied by the fat body. Approximately 1,000 specimens were classified by this method. This is the largest number of specimens ever collected during the winter months for a critical study of winter metabolism and activity of Anopheles in this region.

The data collected indicate that the life span of some female Anopheles is greatly prolonged in the winter months. The absence of males in all collections indicate that they are very intolerant to cold. Collections for Anopheles larvae were made during this study and the information obtained indicates that larval production stops when the temperature remains below 50°F. for very long. Adult females are present in significant numbers at this time but gradually become fewer throughout the winter. The females are active during the warmer periods of the winter and at least a portion obtain a blood meal. Subsequently eggs develop to maturity and it is most likely that they are oviposited. The eggs do not hatch until water temperatures become warm enough. When this occurs the larvae develop. Within a

there time the newly emerged individuals are able to supplement the overwintered population and the first spring generation emerges. This would account for the apparent "explosiveness" with which the spring population usually appears.

The significance of these observations, if further studies prove them to be correct, is obvious. If an insecticide is applied to permanent ponds during the winter to destroy the overwintering eggs much less effort need be expended later to control subsequent larval populations.

3. Hydrological Investigations

When malaria investigations were begun in the Field Station even an attempt was made to assemble available information concerning studies of water resources, natural reservoirs, precipitation and other factors which might influenced the presence of ponded areas when they would be important sources of malaria-carrying mosquito production. Very little material was available since few comprehensive studies have been made of the hydrology of limestone terrain. The program which has evolved from limited observations, which were designed to collect routine climatological data, is considerably more detailed than contemplated originally. It has been developed, however, as a result of the need for the information now being collected and has important relations to many problems not immediately concerned with malaria. As far as malaria is concerned, this phase of the Field Station work represents the first attempt to interpret the occurrence of malaria in terms of hydrological conditions.

The obvious end point is the persistence of surface reservoirs (ponds) at levels where malaria-carrying mosquitoes are produced during the season of malaria transmission. Hence it is necessary to measure the factors responsible for the existence of the reservoirs and to determine their interrelation. A discussion of the hydrological cycle is beyond the scope of this report.

It should be noted that to obtain the desired information measurements of stream-flow, ground-water levels, rain-fall, atmospheric temperature, humidity and other factors are necessary. Those data have been collected since 1939. At present, stream-flow is measured at seven gaging stations, ground-water at ninety observation wells, pond levels at fifteen ponds, rainfall at eight stations, temperature and humidity at three stations. Many of these data are obtained with recording instruments which require attention only once a week. At some locations daily observations are made to provide more detailed information. Tending recording instruments and making measurements at other stations requires almost full time of one engineering aide. Methods of collecting hydrological data have been outlined in detail in previous reports and will not be repeated here.

As work progressed, changes were made in the number, type and place of observations as indicated by analysis of the accumulated data. The first observations were made over a large area and later, as need for more precise information developed, intensive data were obtained from a comparatively small area. During the period covered by this report detailed observations were made at individual ponded situations in addition to the other areas.

For this work four ponds representative of the types which occur in the region were selected for intensive study. A continuous water level recorder, recording rain gage, and water temperature recorder were installed in each pond. On the bank of the pond, away from the influence of the water surface, recording temperature and humidity apparatus was installed. Records are obtained for all losses and gains to these ponds and information collected will indicate the role of ponds as water storage reservoirs and the factors which are responsible for ponds holding water when Anopheles breeding can occur. It would appear that a high degree of precision has been employed for determina-

view of rather gross relationships. Observations have elicited information which indicated that the balance of factors is rather delicate and that a high degree of precision must be employed to supply the needed information.

Compilation and interpretation of these data require specialists in the field of hydrology. As indicated previously when these observations were begun a comprehensive hydrological program was not anticipated. When it became apparent that highly technical work would be involved the assistance of the Water Resources Branch of the U. S. Geological Survey was solicited. This agency assisted with the formulation and establishment of the hydrological program and has made compilations and analyses connected with this phase of the work. Analysis of the mass of data collected during the period of curtailed activity present a difficult problem. It was necessary that experienced hydrologists devote full-time to this task and that proper assistance be available for routine tabulations and plotting. It was further necessary that a complete appraisal be made of the hydrological program to insure that the desired data were being obtained. This work would not have been practical as an undertaking of the Field Station without outside assistance. After negotiations a cooperative agreement with the U. S. Geological Survey was made through the Division of Mines, Mining and Geology of the Georgia Department of Conservation. Under terms of this arrangement the District Engineer of Water Resources Branch furnished personnel to work on the Field Station program within the limits of a stipend provided by the Field Station and matched with an equal amount of Federal funds. Work remained under the direction of the Field Station but activities pertaining to the hydrological work was conducted almost exclusively by U. S. Geological Survey personnel. Through this agreement it has been possible to obtain experienced professional personnel for the hydrological work.

by this arrangement analysis has been made of all hydrological data collected to date and this has been prepared in a comprehensive report at the Field Station. Additional gaging structures have been installed and modifications made in many phases of this part of the program.

STATUS OF THE PROJECT

Five articles for publication in scientific journals are in manuscript or in process of preparation.

1. Winter Activity of Anopheles Mosquitoes.

2. Winter Activity of Culicine Mosquitoes.

3. Marking Anopheles Mosquitoes with Fluorescent Compounds.

4. Observation on Malaria Morbidity in Southwestern Georgia over a Six-Year Period.

5. Hydrological Relationship of Ponds in Southwestern Georgia.

Further analysis of data already collected will probably supply other information which should be published.

Observations on the long-range objective of determining the relation of natural conditions to the occurrence of malaria have been continued as outlined in previous reports. Two factors over which we have no control have prevented accumulation of much positive data. These are the complete absence of malaria infections and the great reduction in the number of mosquitoes. Data related to the occurrence of malaria and mosquitoes have been continued during this remission in anticipation of subsequent malaria transmission. If malaria does return, and there is little reason to believe that it will not, these data will provide an extremely valuable record of the inception and increase of the occurrence. The data are also applicable to many problems other than malaria.

All morbidity data have been compiled and brought down to date. These records will be kept up currently in the future. Unless unforseen conditions again occur these data should always be ready for analysis.

Work on biology of local Anopheles has progressed as indicated in report of activities. Many problems concerned with collecting, sampling of Anopheles populations, and flight range and dispersion are under way. Methods have been developed for more effectively handling Anopheles in studies of this type. Some inconvenience has resulted from moving and, recently, from lack of an insectary for some experimental work. Many field observations on Anopheles biology must be checked under controlled laboratory conditions. This is especially true of many of the problems which have been brought to attention by work on over-wintering studies. It is desirable that a larger more complete insectary be built for these studies.

Hydrological data collected during the past five years have been tabulated and analysed. The program has been modified to permit better accumulation of information related to problems under investigation at the Field Station. Additional gaging structures have been installed at six locations. The expansion of this phase of the work has created the need for a hydrologist to be in residence at the project. Two engineers from the U. S. Geological Survey were stationed at the Field Station on July 1, 1945. This arrangement will enable considerably more activities to be conducted and will afford more effective use of the data being collected.

All of the major hydrological installations have been made with exception of an evaporation station to be installed in the vicinity of the laboratory. Instruments for this installation are already in hand and the station will be placed in operation as soon as instrument shelters can be obtained. It is not anticipated that appreciable additions will be made to the hydrological program in the near future.

Staff at the Field Station includes the following persons at present:

* M. B. Coggins, Jr. (Half time)	Biologist (Director)
* Lt. John W. Zahrt	Entomologist
* Robert L. Lofton	Scientific Aide
* Kenny E. Reynolds, Jr.	Engineering Aide
Mrs. L. B. Bush, Jr.	Nurse
Victoria Thompson	Nurse
* Marjorie Vines	Clerk
** L. R. Mills, Jr. (One-fourth time)	Engineer
** E. L. Hendricks (Three-fourths time)	Engineer (Hydrologist)

* U. S. Public Health Service

** U. S. Geological Survey

In addition two laborers were employed most of the time and a carpenter for the month of August. These were paid by the Field Station.

Special mapping and architectural work was accomplished by personnel detailed from the U. S. Public Health Service. At least one man year was devoted to this work.

SUGGESTIONS FOR FUTURE WORK

Sept. 1, 1945 - Aug. 31, 1946

Much of the Field Station activity is concerned with the long-term program investigating the natural history of malaria. Consequently, a large proportion of work will be devoted to pursual of this problem. This objective is regarded as the primary, all-inclusive problem which serves to collect and relate all phases of Field Station activity. There are several other epidemiological problems which might be as easily investigated by the same methods that malaria morbidity data are obtained. Likewise, any of several insect-borne diseases could be investigated with the same biological methods that are applied to mosquito problems. The hydrological work is intimately related to many problems of agriculture, public health, and industry.

It will be noted from the publication of the Field Station staff and from the titles listed in this report, that the secondary problems which developed in connection with investigation of more comprehensive ones, have a direct bearing on the investigative program as a whole. The program is designed to continue supplying these portions of the picture to contribute to the entity of the main problem. It is believed that the selection of a primary objective, especially one as inclusive as the natural history of malaria, provides the best means of assuring coordination and continuity of research.

Specifically, the following proposals are made for the next fiscal year.

1. Epidemiological Investigations

Continue morbidity studies by house visits and surveys as in the past; conduct observations in the Atabrine prophylaxis area; maintain public health work.

In view of expansion of typhus fever control facilities and investigation program to be established in adjoining counties, an effort should be made to investigate typhus fever morbidity and potential with the idea of cooperating with the U. S. Public Health Service if an opportunity develops.

2. Ecological Investigations

Continue investigation of Anopheles biology to determine variations in larval and adult populations, if they become measurable. With installation completed this year very extensive temperature and humidity data will be available. Larval development and adult mosquito activity should be intensively studied in relation to these factors.

Work should be continued on the improvement of methods of Anopheles collection and methods for making precise measurements of adult Anopheles populations. Flight range and dispersion studies should be continued. These problems would involve use of marking technics which should be studied further during the winter months.

Additional data should be obtained by winter observations of Anopheles. The possibility of variations in winter and summer eggs should be studied from the standpoint of structure and rapidity of hatching under a variety of conditions. These studies should be observed under laboratory as well as field conditions.

Blood feeding habits of Anopheles should be thoroughly investigated if populations are high enough to permit such studies to be made. If this is possible, the D D T experimental spray program originally proposed for the past year should be conducted. This is extremely important since the DDT residual spray programs in operation for malaria control throughout the country are predicated on the assumption that malaria is transmitted by mosquitoes which feed on humans inside houses. If malaria-carrying mosquitoes do not

enter houses in connection with most malaria transmission, there is little basis for the program. No information is available on this subject and it is urgently needed at present.

A great deal of information is needed on the flora of Anopheles breeding places to fill a gap in investigation of Anopheles biology. This work must necessarily be done by an experienced botanist. Such men are at present employed by the U. S. Public Health Service and an effort will be made to obtain the services of one for the Field Station. A year would be sufficient for the accumulation of most of the desired information and the necessary supplemental work could be done during subsequent summers by visiting botanists.

3. Hydrological Investigations.

Routine measurements in progress should be continued as during the past year unless modification is indicated during the course of observations.

Comprehensive studies on evaporation from standard stations and from pond surfaces should be undertaken. Very little is known about evaporation and its measurement. With the installations made in the four ponds selected for intensive study, these ponds are essentially evaporation stations. Records from these installations, together with those from the installation at the Field Station, should provide a valuable contribution to knowledge of evaporation measurement and its importance in the hydrological cycle.

The significance of ponds as reservoirs for water storage should be studied intensively not only from the point of view of their existence during mosquito breeding season but also from the standpoint of agricultural economy. This means of surface storage probably plays an extremely important part in conserving water for plant growth. If this is proven to be the case, extreme caution should be exercised in the elimination of ponded areas.

A complete geological description is needed of the area in which activities are being conducted. This is essential for reporting accurately and factually much of the information being collected. This problem will be discussed with the State Geologist and it is possible that this information can be obtained by one of his geologists.

Additional detailed survey work is indicated in some of the ponded areas where intensive investigations are being made. Arrangements were made last year for U. S. Public Health Service engineers to be assigned during the winter months for this purpose. It is likely that the same arrangements will be possible during the next winter.

Proposed Budget

Emory University Field Station

Sept. 1, 1945 - Aug. 31, 1946

A.	Personel services		$8200.00
	1. Nurse's salary and travel (Mrs. I. B. Bush, Jr.)	$2400.00	
	2. Nurse's salary and travel (Victoria Thompson)	2100.00	
	3. Incidental labor	1200.00	
	4. Technical assistant (3 months temporary)	300.00	
	5. Travel for director not reimbursed by U.S.P.H.S.	200.00	
	6. Stipend to U. S. Geological Survey	2000.00	
B.	Non-expendible equipment		$1500.00
	1. General station, library, and office equipment	1000.00	
	2. Laboratory equipment	250.00	
	3. Field equipment	250.00	
C.	Expendible supplies and service		$1800.00
	1. Laboratory supplies	500.00	
	2. Field supplies	200.00	
	3. Office supplies	150.00	
	4. Engineering and mapping supplies and map printing	100.00	
	5. Nursing supplies	100.00	
	6. Truck maintenance	450.00	
	7. Heating and power	300.00	

D.	Contingency and maintenance fund	$1500.00
E.	Building fund	$ 900.00
	Total	$13,900.00

Expenditures by Federal Agencies

at Emory University Field Station

Sept. 1, 1945 - Aug. 31, 1946

U. S. Public Health Service		$17,654.00
1. Director's salary and travel (Half time)		
M. H. Goodwin, Jr.	$3000.00	
2. Entomologist's salary		
Lt. J. W. Zukel	3000.00	
3. Scientific aide's salary		
R. L. Lofton	2650.00	
4. Engineering Aide's salary		
H. E. Reynolds, Jr.	2650.00	
5. Clerk's salary		
Marjorie Vines	1704.00	
6. Engineer's salary (5 months)	1400.00	
7. Transportation and automotive maintenance (65 vehicle months)	3250.00	
U. S. Geological Survey		2,000.00
1. Matched fund for stipend		2,000.00
Total		$19,654.00